FORENSIC EXAMINATION
OF
BLOOD AND BLOOD STAINS .

DOUGLAS KERR, M.B., ChB.

M. D. 16th Dec., 1927.

FOREWORD

Although the examination of blood stains forms an important part of Forensic Laboratory work, text books only mention routine tests for stains to which at the most, two or three pages are given. Examination of blood other than stains is never considered.

Being brought into contact with cases calling for examination beyond the usual routine, I have turned in vain for aid to text books of Forensic Medicine, Toxicology, Chemistry and Physiology. Especially in the case of Physiology was I led astray at first, as the descriptions there relate to pure substances which are not encountered in Forensic Medicine.

Whilst cases requiring investigation of uncommon forms of haemoglobin are few, when they occur it is not merely a description of the compound, but minute details and references which are required. I have endeavoured to supply this. Each statement and test has been carefully examined by me before being accepted; save a few cases where the reverse is stated. Some of the recognized tests have been the object of original investigations, and in almost all

cases experimental enquiries have been made into their comparative value and fallacies. Details of these researches are not given here, the results having been summarized. Wherever I have stated my opinion it is based on practical experience and investigations.

Enquiries into the subject of blood groups are still progressing, but I am not aware of any other work in English dealing with the present position as here summarized.

Owing to its present prominence, the examination of Alcohol in the blood has been included.

An Appendix describes Carbon Monoxide poisoning, to an investigation of which I devoted many months last year.

Whilst I have endeavoured to present in this Thesis a complete survey of Forensic blood work which a Medical jurist will find of value, it has also been my aim to make this understandable to one commencing the study of the subject.

September 1927.

CONTENTS

ILLUSTRATIONS.

INTRODUCTION

It is only from an understanding of the chemistry of haemoglobin and its derivatives that a clear idea of the value and rationale of the various tests for blood can be obtained.

From this an observer is able to recognize what compound he is dealing with, and what further confirmatory tests are applicable. The derivatives of haemoglobin are considered in detail under that heading, but a brief description of them is necessary before considering any other aspects of the subject.

Haemoglobin is contained in the red blood corpuscles, and only appears in the serum in cases where the corpuscles are haemolysed. It has the property of readily taking up oxygen, forming a loose combination - oxyhaemoglobin. All freshly shed blood whether of venous or arterial origin, is in the state of oxyhaemoglobin, as on exposure to the air the haemoglobin immediately takes up oxygen.

The combination of oxygen and haemoglobin is loose. The oxygen in it is readily removed by the tissues, or by reducing agents converting it into haemoglobin, often referred to as reduced haemoglobin, although this is a misnomer, the correct term being either haemoglobin, or reduced oxyhaemoglobin. Haemoglobin itself is a conjugate protein, that is it

consists of a complete protein molecule globin, combined with a non protein molecule, haemochromogen, the latter contains all the iron of the haemoglobin. When haemoglobin is decomposed in the absence of oxygen these two substances globin and haemochromogen are formed*, haemochromogen is however very readily oxidized to a more stable compound, haematin. So when oxyhaemoglobin which is the usual compound found in fresh blood, is decomposed, globin and haematin are the resulting substances.

This decomposition may be brought about by heat in which case the albumen (globin) is coagulated, or by hydrolysis with weak acid or alkaliwhen the globin remains in solution. Depending on whether the

* This explanation of the composition of haemoglobin is retained here as it explains in a simple manner the sequence of changes. Anson and Mirsky(69) however state that haemochromogen is itself a conjugate protein, in reality globin haemochromogen, and that the globin may be replaced by other proteins, amino acids, ammonia, hydrazine hydrate, pyridine,and many other nitrogenous bases, giving pyridine haemochromogen, etc,. These different haemochromogens though giving the same spectra differ very slightly in the position of the bands as is well exemplified in the case of cyanhaemochromogen (q.v.) and haemochromogen (globin).

reaction of the medium is acid or alkaline, we obtain the respective haematin, termed by some authors acid or alkaline oxyhaematin in contrast to haemochromogen which they term reduced haematin.

Haematin may be converted from acid to alkaline or vice versa by altering the reaction of the medium.

Acid haematin crystallizes out in the presence of a chloride (usually present in sufficient amount in the blood itself) as haematin hydrochloride or haemin, giving the socalled haemin crystal test.

Alkaline haematin in the presence of a reducing agent, is converted into a cherry pink substance, reduced alkaline haematin or haemochromogen.

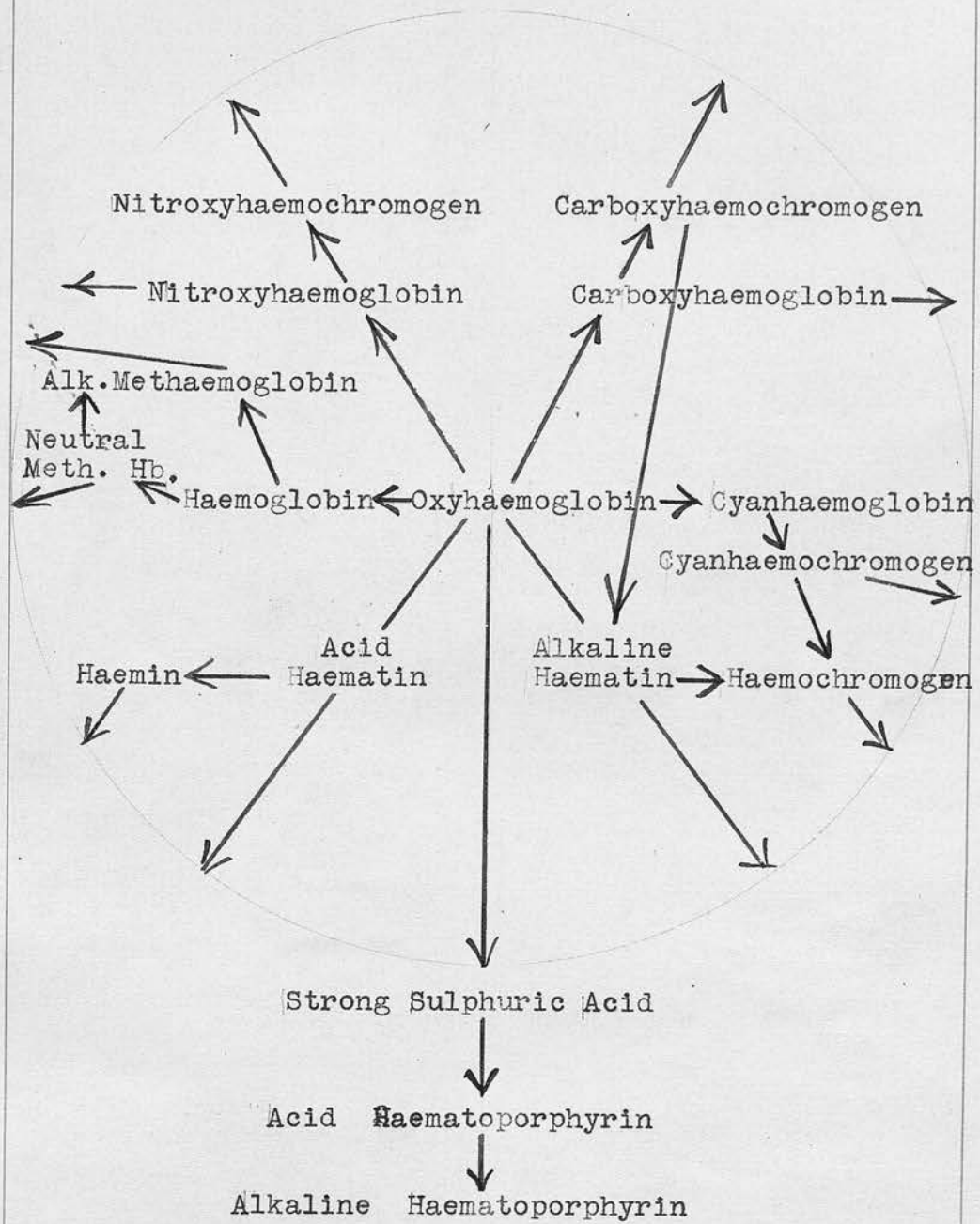
Haemochromogen can be made to crystallize out and forms a further test for blood (q.v.41). On oxidation haemochromogen is reconverted into alkaline haematin. The decomposition can be carried further by more vigorous meams, such as treating with strong sulphuric acid, when the iron is split off from the haematin, and an iron free compound - haematoporphyrin - related to the bile pigments is formed, the iron being converted into ferrous sulphate. For this it is not necessary for the haemoglobin to be first converted into haematin; the addition of strong sulphuric acid to any iron containing derivative of haemoglobin, being all that is necessary for the production of haematoporphyrin.

Haematoporphyrin, occasionally referred to as iron free haematin, exists in both acid and alkaline form, according to the reaction of the medium. It can be reconverted into haematin, that is, the iron may be replaced, by suitable treatment with ferrous tartarate in ammonia, though this procedure is difficult.

Haemoglobin forms combinations with gases such as oxygen, carbon monoxide, nitric oxide, carbon dioxide and sulphuretted hydrogen; the usual one being oxy-haemoglobin. It may however, combine with oxygen in a more stable form to that found in oxyhaemoglobin, forming a compound methaemoglobin which is found in old decomposing blood, or blood exposed to air and light for some time. Methaemoglobinexists in neutral and alkaline forms depending on the reaction of the medium; on addition of a reducing agent it is converted into reduced haemoglobin.

Blood readily takes up carbon monoxide (in illuminating gas)the oxygen of the haemoglobin being replaced by the carbon monoxide, forming carboxyhaemoglobin. In a similar way under appropriate conditions a compound with nitric oxide - nitroxyhaemoglobin - is formed. These gases also produce compounds with other derivatives of haemoglobin, giving carboxyhaemochromogen, nitroxyhaemochromogen, etc.

DIAGRAM TO ILLUSTRATE THE RELATIONSHIP OF HAEMOGLOBIN DERIVATIVES



EXAMINATIONS OF BLOOD STAINS

General considerations

Blood stains may vary from bright red through pale yellow brown to dark brown or black, and on dark coloured material are often hard to see, in such circumstances they are often rendered more visible by artificial light. By clotting and coagulation they stiffen the material stained, and if on woollen or a similar material cause the fibres to become matted together. On steel or hard surfaces they shew as dark shining spots which are often fissured and cracked. Diagnosis from naked eye examination is usually simple in large stains, but smaller stains frequently present difficulties.

Blood stains vary in shape, extent, colour and character, and valuable information may frequently be obtained from a naked eye examination of the stain

POSITION. The position should be noted as this may refute or confirm an accused person's statement, and special attention should be given to this in the examination of blood stained garments. Where the garments have been saturated with blood it is sometimes possible to determine from which side of the garment the blood has originated by observing the presence of clotted blood on the side of the incidence. On the other hand, a garment may be so saturated that both sides drip blood; in such a case clots may be found on both surfaces.

BLOOD STAINS

Fig. 1. From spurting blood.

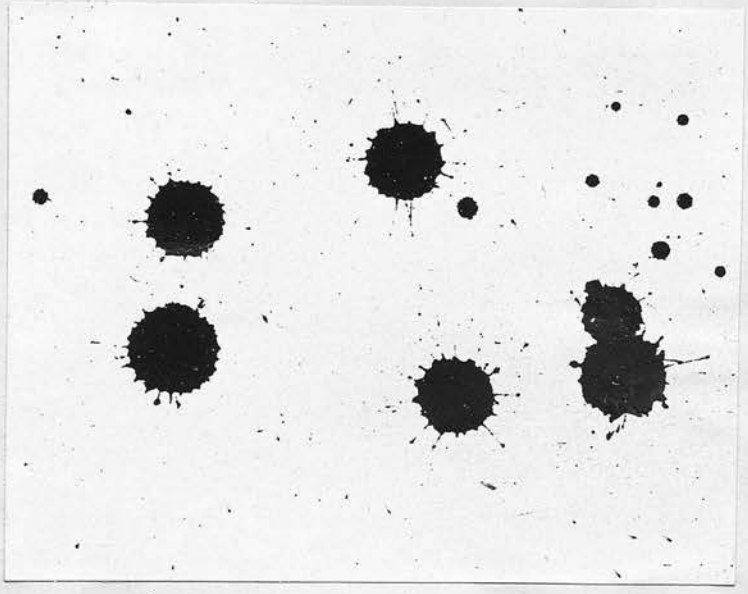


Fig. 2. Drops falling vertically.

Splashes,-Splashes may indicate the direction from which the blood has come. The blood tends to become piled up at the part of the stain distant from the source, in some cases even overflowing and forming a smaller stain or streak giving the appearance of a mark of exclamation. (Fig. 1). When however the surface splashed is not horizontal, though the shape of the splash is maintained, the blood tends to accumulate at the lowest part of the stain, and the smoother the surface the more noticeable this is.

Blood which has fallen vertically on to a surface causes roundish stains with subsidiary splashes in all directions. (Fig. 2.)

Although arterial blood spurts, it cannot be diagnosed from venous blood from the fact that it has caused a series of spurt like splashes, as venous blood may accumulate round a wound and then by some sudden movement be flung away, giving splashes similar to those produced by a spurting artery.

Colour of Stains, - A stain caused by freshly shed blood will be bright red in colour from the presence of oxyhaemoglobin. This will be the case whether the stain was caused by arterial or venous blood, as in the latter case the blood is reoxygenated by exposure to the atmosphere. In a short time the colour of the stain changes varying from light yellow in faint stains, to brown, or black in deeper stains,

owing to the formation of either methaemoglobin, or haematin. This depends on the existing conditions, a dry atmosphere favours methaemoglobin, but this may later be converted into haematin. Exposure to sunlight, heat, treatment with chemicals, and the presence of perspiration on stained garments increases the rapidity with which this takes place. Sorby (45) found that in the centre of the town (Sheffield) the change was brought about in a few hours, whilst in the country and away from houses, it required at least a week to produce any appreciable result.

AGE.- It is not possible to estimate the age of a stain by a consideration of the part played by the above mentioned factors in any individual case, ~~that~~ but it may be accepted as a general rule that, WHEN THE COLOUR OF A STAIN IS BRIGHT RED," IT IS PRESUMPTIVE PROOF THAT THE STAIN IS OF RECENT ORIGIN. ON THE OTHER HAND IF IT IS BROWN, IT IS NO PROOF THAT IT IS OLD. 'Recent' in this case, may mean within ten days, but more probably within six.

Attempts have been made to estimate the age of a stain by noting its solubility. Pfaff constructed a scale based on the solubility of the stain in 1/120 solution of arsenious acid. Fresh blood is said to dissolve at once. Blood two to three days old in 15 minutes, in 30 minutes and so on. Tamassia (46) prooved conclusively that this scale was quite useless. A summary of Tamassia's results is given by Sutherland.

~~Sutherland~~. THE ONLY OPINION THAT CAN BE GIVEN REGARDING THE AGE OF A STAIN IS,(a) THAT IT IS RECENT IN ORIGIN OR, (b) THAT THERE IS NO EVIDENCE THAT IT IS OF RECENT ORIGIN.

DRYING AND COAGULATION.- The rate of coagulation and drying of blood may give rise to erroneous conclusions as to the time a crime was committed; as for instance, where only blood stains are found, or where an assaulted person is unconscious and dies later from his injuries.

The time of coagulation of human blood is about three minutes, but this does not mean that at the end of three to five minutes the drop or pool of blood has become jellylike or solid. Coagulation occurs so quickly that it is unlikely to be of medico-legal significance. If a probe or similar object is drawn through a pool of blood on the ground, about four or five minutes after being shed, it will be seen to leave a line in the fluid only slowly obliterated. Coagulation has taken place, yet the blood is still qite fluid. If a pool about eight inches in diameter is observed, a scum will be seen to have formed on the surface after about one and a half to two hours. About ten to fifteen hours will elapse before the stain is quite dry.

These rates are only approximate, and depend on the depth and area of the blood. A large pool may

be days before losing its fluid character and becoming sticky or dry. On the other hand, a single small drop will be quite dry in an hour. A spot 1/3 inch in diameter took 3½ hours to dry.

ROUGHLY THEN, SMALL SPOTS ARE DRY IN AN HOUR OR TWO, LARGER AREAS BECOME COVERED WITH A SCUM IN ONE TO TWO HOURS, WHILST THE TIME THAT ELAPSES BEFORE THE STAIN IS DRY DEPENDS ON THE SIZE AND DEPTH OF THE STAIN, AND VARIES FROM ABOUT SIX HOURS TO DAYS.

The temperature and the surface on which blood is shed is stated to affect the rate of drying and coagulation. On comparing the influences of different surfaces and materials on the rate of drying and coagulation, I found the differences slight. As the times given above are wide, this factor need not be considered. Extremes of wind, humidity, or temperature, as in the case of most fluids will have an influence on the rate of drying.

SOLUBILITY.- Before any tests can be applied to a blood stain, some of the blood colouring matter must be brought into solution, or the clot dissolved until it is so thin that it allows enough light to pass to give the spectroscopic tests. The solubility then of a stain beeomes of great importance, as does also a knowledge of the derivatives of haemoglobin which result from the use of the different solvents. It is affected by the age of the stain, the amount of exposure to light, heat or air, or by chemical treatment

the stain may have received either in attempts to remove it, or by contact or contamination with chemical substances in the stained material.

Most text books on Forensic Medicine give details of the solubility of stains under various conditions. These details are interesting, and will be referred to later. The practical application however, of these investigations is not made clear, usually beyond the reader being left with a recommendation to use such and such a solvent.

Blood when shed from the body is readily soluble in water. By exposure the haemoglobin is converted into methaemoglobin, likewise also soluble in water. By long exposure, by heat, or the action of chemicals, it is further changed into haematin which is almost insoluble in water, or by vigorous treatment into haematoporphyrin which is also insoluble in water.

In other words, A BLOOD STAIN IS EITHER SOLUBLE IN WATER, IN WHICH CASE WE ARE ABLE TO MAKE THE USUAL ROUTINE EXAMINATIONS, AS IN THE CASE OF FRESH BLOOD, OR, IT IS INSOLUBLE, THERE HAVING FORMED HAEMATIN OR MORE RARELY, HAEMATOPORPHYRIN, AND WILL REQUIRE SOLVENTS OF THESE SUBSTANCES.

In the case of haematin,(q.v.p.130) it will be soluble in dilute alkali, (sodium hydroxide) or dilute acids, giving a solution with which we carry on the routine examination. In the rarer instances where the

treatment of the stain has resulted in the formation of haematoporphyrin, the stain will be soluble in any solvent of haematoporphyrin, and the presence of blood is proved by obtaining acid and alkaline haematoporphyrin as described under spectroscopic tests.

If more information is required than the proof of the presence of blood, that is, if it is necessary to perform the precipitin test for the determination of the source of the blood, or to group the blood, further factors come in to play, and these are determined described when dealing with thetests in question.

EFFECT OF HEAT.- HEAT BELOW THAT OF BOILING WATER HAS LITTLE EFFECT ON THE SOLUBILITY OF A STAIN IF TIME IS ALLOWED FOR THE SOLVENT TO ACT, THOUGH IT MAY HASTEN THE FORMATION OF METHAEMOGLOBIN.

Katayama (42) found that blood dried in porcelain dishes and exposed to a temperature of 80° C for an indefinite time or up to 100° C for one hour, were soluble in water, and if heated to 140° C it was still soluble in NaOH or glacial acetic acid within 24 hours

Hammerl (49) found that after heating to 135° C to 143° C blood no longer gave the guaiac test, and at 145° C lost the power of forming haemin crystals. My own observations agree with these findings.

The effect of heat on the precipitin reaction is discussed under that heading.

EFFECT OF SUNLIGHT.- In the tropics difficulty is sometimes experienced in dissolving stains. This is due to the action of the sun and not to the actual heat. Hammerl (49) found that after 3 days exposure, the stain was insoluble in water, after 16 days, was insoluble in potassium cyanide, and after three weeks no Teichmann's crystals could be obtained, but the stain still yielded an extract for spectroscopic purposes when treated with glacial acetic acid, or strong sulphuric acid. He also found that as in the case of heat, stains on hard surfaces are but little affected by sunlight. I have not been able to check these statements.

DIFFICULTIES DUE TO AMOUNT, INSOLUBILITY, OR CONTAMINATION OF STAINS.

FAINT DIFFUSE STAINS.- It is quite easy by means of the preliminary chemical tests for blood (q. v.) such as the guaiac or phenolphthalein tests, to come to the conclusion that blood is present in a faint diffuse stain, but this is a long way short of proof.

To perform the conclusive tests for blood, a certain concentration of haemoglobin is necessary, and this may be hard to obtain when attempts have been made to remove the stain by washing.

By taking a large area of the stained material and soaking in water, a very dilute solution of blood may be obtained, so dilute that no conclusive tests

will give a positive result. The blood may however, be concentrated by precipitating it from its aqueous solution by means of sodium tungstate.

Petterson and Haines (50) consider this procedure of great value, and their technique is given below

I find that with practically colourless solutions of blood which, if sufficient depth of solution be employed, will still give a very definite result with the microspectroscope, I am unable to obtain either the haemin or haemochromogen crystal tests with the resulting precipitate. With stronger solutions or stains which give satisfactory results with this method, blood is more easily PROVED present by other means. It may however, be tried as a last resort.

SODIUM TUNGSTATE.- Precipitates the blood from its aqueous solution; the precipitate may be tested by the haemin or haemochromogen test, or by means of the spectroscope.

A piece of cloth- the amount required depending on the dilution of the blood- is removed and placed in an evaporating dish containing distilled water in which is dissolved a crystal of potassium iodide. The fabric is allowed to macerate, and the process repeated two or three times. The fluids are collected and acidulated with acetic acid and filtered, then treated with a saturated solution of sodium tungstate, also acidulated with ~~with~~ acetic acid; as much as 5

or 10 CC of sodium tungstate may be required if the solution is deeply coloured with blood pigment. (In this case blood could be easily proved by spectroscopic tests). The blood pigment collects at the bottom of the tube on standing.

If there is not sufficient blood to impart a perceptible colour to the extract, the addition of sodium tungstate will only cause slight turbidity. In this case, the mixture should be boiled and put aside for a day or two to settle when the blood pigment will be found in the precipitate at the bottom.

INSOLUBILITY.- As in the case of old or heated stains already referred to, difficulty may arise on account of insolubility of the stain in cases of stain on such substances as leather, oak, and other materials which contain tannic or other acids.

It is also said (Buchanan 54) that with stains on dyed fabrics which have been mordanted, the mordant may fix the stain. I have not found this to be the case. Mordants such as alum, potassium bichromate, stannous chloride etc, will coagulate and so fix the stain when applied direct to blood; but unless excess of mordant has been used and left on the stained garment, the mordant itself has been fixed by the dye and has no action on the blood. The insolubility is easily overcome when it is remembered that the action of these acids is to convert the haemoglobin into haematin.

ATTEMPTS AT REMOVAL.- One of the best methods for removing blood stains is by prolonged maceration in cold water. Boiling water tends to fix the stain by coagulating the albumen, but I have been unable to detect blood after washing a stained handkerchief with soap and hot water.

Efforts are often made to remove stains by treatment with chemicals such as ammonia. TREATMENT EXCEPT BY WATER, USUALLY ONLY TENDS TO FIX THE STAIN AND RENDER IT MORE PERMANENT BY CONVERTING IT INTO HAEMATIN OR HAEMATOPORPHORIN.

Petterson and Haines (50) state, that in stains treated with naphtha or benzene and certain other similar substances, the blood pigment is so decomposed that it cannot be detected by the ordinary chemical tests. With this I fail to agree. I have found that whilst blood stains on cloth cleaned with petrol or benzene bensole until no evidence of staining was visible, sometimes fail to give haemin crystals, they still react to the haemochromogen crystal test and give the benzoidine reaction.

STAINS MISTAKEN FOR BLOOD.- INSECT STAINS.-

Sutherland discusses the question of stains caused by crushing blood sucking insects, and states that in 10 per cent of cases he was unable to discover tracheae, bristles, or other evidence of the insect, and that haemin crystals and haemochromogen may be obtained from these stains.

I have obtained both these tests with the blood still in situ, in the stomach of the flea, and in Fig (4) shews haemin crystals in the stomach of the flea.

WE MUST ADMIT THEN, IN CERTAIN CASES OF SMALL BLOOD STAINS, THAT THESE STAINS MAY HAVE BEEN CAUSED BY FLEAS OR LICE, EVEN THOUGH WE FIND NOTHING BUT THE EVIDENCE OF BLOOD.

In these cases however, each individual stain should not exceed about 3 m m in diameter, and the position and distribution on the clothing together with the number of stains present, the general condition of the garment and the social status of the individual, must all be taken into account.

RUST.- These stains are insoluble in water, but soluble in dilute hydrochloric acid, giving the reactions for iron. These reactions are not given by haematin, and so serve to distinguish the two.

HAEMIN CRYSTALS IN STOMACH OF FLEA.

(From a specimen in Prof. Littlejohn's collection)

Fig. 3. Very low power

Fig. 4. (x 75)

FAECES.- On account of their colour faecal stains may be suspected of being due to blood, and I have examined shavings taken from a lavatory seat stained in this manner which it was suggested had been stained by blood. Faecal contamination of clothes sent for examination is of frequent occurrence.

Normal faeces may give a positive result with the preliminary tests for blood, such as the benzidene and phenolphthalin tests, and these should only be used as indicators for further examination.

Microscopical examination reveals the presence of the usual constituents of faeces muscle fibres, connective tissue fibres, starch granules, vegetable detritus, crystals, yeasts, and sometimes blood corpuscles.

OTHER STAINS.- A large number of other substances may cause stains which resemble those caused byblood in appearance. The tests for blood are now so certain and delicate that a detailed consideration of these stains is unnecessary. I have however, been asked by counsel, whether stains due to such and such a substance could simulate blood stains in appearance.

The following stains amongst others may simulate blood stains. Egg, red paint, oil or grease spots on dark clothes, tar or pitch spots on seamen's garments, iron mould, fruit stains, anatto,(used in the milk trade) and according to Sutherland, saliva ejected during betel chewing.

VEGETABLE MATTER.- Ammonia changes stains due to vegetable matter, fruit stains etc, to green, black or crimson, leaving blood unaffected.

ANILINE DYES.- Are turned yellow by nitric acid which leaves blood unaffected. These dyes are now being used in the milk trade.

INDICATORS FOR THE PRESENCE OF BLOOD

(Preliminary Chemical Tests)

Articles submitted for examination in connection with a medico-legal case are often numerous, in a dirty condition and covered with all kinds of stains

As a rough means of selecting stains which may repay further examination, certain preliminary chemical tests may be employed.

THESE TESTS SHOULD NEVER UNDER ANY CIRCUMSTANCES BE TAKEN AS EVIDENCE OF THE PRESENCE OF BLOOD, AND SHOULD NOT EVEN BE USED AS CONFIRMATORY TESTS IN MEDICO-LEGAL WORK.

They give positive results with various substances mentioned below, and are only to be used as a pointer to the stains to be examined. As a negative test they are occasionally of use, that is, if they prove negative. The stain is most probably not due to blood. They consist of tests which are used for the most part, in clinical medicine as tests for occult blood, and it cannot be too strongly emphasised that they are not medico-legal tests in the sense of proof for blood. When one finds it stated in a medico-legal report that these tests proved faintly positive, it is time they were removed from under the heading of 'tests'. It is for this reason that they are here considered under the heading of 'indicators'

Of these tests, those most commonly in use are the guaiac, hydrogen peroxide, benzidine and phenolphthalein tests, of these the last two are the best.

GUAIAC TEST

(Van Deen's, Day's, Schönlein's). When a drop or two of fresh tincture of guaiac (1gram guaiac resin in 60 CC absolute alcohol) is added to a solution containing blood, a white precipitate forms, if to this a little hydrogen peroxide is added, a blue colour appears at the junction of the fluids.

A frequent mistake in performing this test is the use of a too concentrated solution of guaiac which causes a thick white precipitate when added to aqueous blood solutions.

The test may be performed by moistening a stain with water, then pressing on the stain a piece of filter paper (or by using filter paper through which the solution has passed). To the filter paper is added a drop of the tincture of guaiac, and a drop of hydrogen peroxide, ozonic ether or oil of turpentine, a blue colour appears almost immediately.

Fallacies, pus, urine,containing iodides, saliva, milk, potassium permanganate, faeces, salts of iron, raw potato pulp, and many other substances may give a blue colouration. It is said (Taylor 47) that these may be eliminated by heating or by observing whether the colour appears before

the addition of hydrogen peroxide or not.

As the only possible use of this test is as a negative test, that is, to give an indication of the stains worth testing for blood, these points do not arise in practice.

Sutherland (9) found that distilled water containing 1 part of sodium chloride in 600 gave a positive result.

Sodium chloride is present in the sweat and urine, and it is hard to see how anyone can speak to the presence of blood from the result of this test.

BENZIDINE TEST.-

In this test a saturated solution of benzidine in glacial acetic acid is used in place of tincture of guaiac. Pus, saliva, mucous, iron salts and iodides also give this reaction. It is to be regarded in the same light as the guaiac test, but is cleaner to handle.

ALOIN TEST.-

(Schaer's). The reagent used here with hydrogen peroxide is 3 per cent aloin in 70 per cent alcohol. A brick colour is produced.

PHENOLPHTHALIN TEST.-

(Kastle Meyer test - Boas's test (58) Utz Meyer test). When alkaline phenolphthalin solution and hydrogen peroxide are added to a solution of the

suspected stain, a pink or red colour is formed almost at once, if blood is present.

Kastle (57) states that the test detects blood in a dilution of 80,000,000. With a dilution of 20,000,000 the reaction is strong, and cannot be mistaken, but above this dilution I find that the reaction tends to get so faint as to be doubtful.

When performed in a test tube, a dilution of 80 to one hundred million can be recognized if the test is made in a $\frac{1000}{cc}$ glass cylinder.

Balthazard (64) states that copper salts are a grave source of error.

I have obtained positive results with water contaminated with copper, strong acids, rust dissolved in hydrochloric acid, healthy faeces and a few other substances. Saliva occasionally gives a doubtful result. As an indicator, it is a good test, but it is so sensitive to contamination that a positive result should be repeated several times, again, the test is so delicate that many garments in daily use by criminal classes may shew a positive result.

These two drawbacks make what would otherwise be a very good confirmatory test a source of danger in medico-legal work. IT SHOULD BE CLASSED AS AN INDICATOR, AND USED AS SUCH. (q v).

The reagent is best prepared by dissolving 1-2 grams of phenolphthalein and 20-25 grams of potassium hydrate in 100cc of distilled water. About 15 grams of powdered zinc is added, and the solution heated until discoloured. This will take about fifteen minutes.

Phenolphthalin is formed owing to the reduction of the phenolphthalein by the zinc. On long standing the solution gradually acquires a faint colour due to oxidization. Glaister (55) recommends that a small amount of zinc should be left at the bottom of the solution to prevent this oxidization.

LEUCHO MALACHITE GREEN.- (Adler's test)

In this case a solution of leucho malachite gre green is used with the hydrogen peroxide, and a green colour obtained with blood. The leucho malachite green solution may be prepared in the following proportions. Basic leucho malachite green, 1 grm,30% acetic acid 30 cc, aqua dest 100 cc.

It is said that the presence of iron salts and nitrates vitiates the test, and I have found this to be the case.

HYDROGEN PEROXIDE.- (Gantter's test).

When hydrogen peroxide is added to blood stained material a reaction takes place and a fine froth is produced. Many animal and vegetable substances give a positive reaction.

PYRAMIDON TEST (AMIDOPYRINE TEST)

This test discovered by Thevenon and Roland is a modification of the Benzidine test and is used for the detection of occult blood.

The method is to add to 2-3 cc of the fluid to be examined, 16 drops of glacial acetic acid, 2 cc of alcoholic solution of amidopyrine (pyridine 5, alcohol 95 % 100), and 12 drops of hydrogen peroxide.

The appearance of a lilac colour is positive for blood. It is said to shew blood in a dilution of 1 : 21000.

MEDICO-LEGAL APPLICATION OF THE PYRAMIDON TEST

Laet (88) states that following his technique this test is as specific as the crystal or spectroscopic tests. I have not made a comparative investigation but from my experience of tests of a similar nature and his own account given below, I very much doubt it.

The reaction serves specially for dried blood spots and ~~as it~~ is not specific for very dilute blood solutions, pus, saliva and oxygen containing water after some time gave a blue tint with alcoholic pyramidon solution. The following is the technique.-

The small blood stain or the blotting paper on which with some dilute acetic acid the material to be investigated has been placed, is snipped into strips three to six mm broad. With a pipette

and without mixing the solutions, 2 drops glacial acetic acid, 2 drops freshly prepared hydrogen dioxide solution, 3 drops of a 10 per cent alcoholic solution of pyramidon are placed in a small test tube. The strips of material with the blood spots uppermost are so dipped in the test tube that the blood spot is in the pyramidon solution, and the lower third of the material in the acetic acid. A positive reaction shews in from one to thirty seconds as blue currents stretching out from the blood spots into the liquid. After some hours the solution becomes brownish, whether it was blue or not.

Done in this way, he states the reaction is negative with pus or saliva, and is recommended by him on account of its simplicity and specific character.

CONCLUSIVE CHEMICAL TESTS FOR BLOOD

There are two conclusive chemical tests for blood. The obtaining of haemin or of haemochromogen crystals. Of the two, the latter is the more certain and the easier to perform.

HAEMIN CRYSTAL TEST.- (Teichmann's test).

This is a conclusive test for blood, and was first described by Teichmann in 1853. As it frequently fails a negative result does not exclude the presence of blood.

When haemoglobin is heated with any mineral or organic acid, it is converted into haematin and if a chloride be present is deposited as brownish crystals of haematin chloride, haemin crystals.

The acid universally employed is glacial acetic acid, the chloride except in the case of old or altered stains, is usually present in sufficient amount in the blood itself. To make certain however, it may be added either by the addition of a small crystal of sodium chloride to the preparation on the slide by using Nipper (41) solution (potassium iodide potassium bromide and potassium chloride $\overline{aa}$ 1 gramme glacial acetic acid 100 cc), or by evaporating some normal saline to dryness on the slide.

I have found the latter method to give the best results.

In the same way, by using bromides or iodides similar crystals of haematin bromide and iodide may be obtained.

Douris (65) mentions a method which he attributes to Strzyouski, in which the iodide is obtained, the solution used being

Alcohol
Glacial acetic acid
Water aa 1 cc
Hydroiodic acid 2-3 drops.

HAEMIN CRYSTALS.-

Whilst the test is usually easily performed, great difficulty is sometimes experienced in obtaining a positive result in the undoubted presence of blood.

FALLACIES.-

Crystals cannot be obtained from blood which has been heated to 140° C or from blood that has been exposed to the sun for a long time. Decomposition, old stains, sun, exposure to weather, washing with alkalis and soap, cleaning with petrol, benzine etc, rust, metals, fats, analine dyes, excess of salt or of absolute alcohol and the presence of water may all have an inhibitory effect.

With regard to the presence of water, most writers insist that unless the acetic acid is glacial the test will fail. This is not the case. Rabinovitch (56) tried various dilutions of acetic acid with water and obtained positive results with dilutions up to 50 % water.

I have not been able to obtain crystals with a greater dilution than 30 % water.

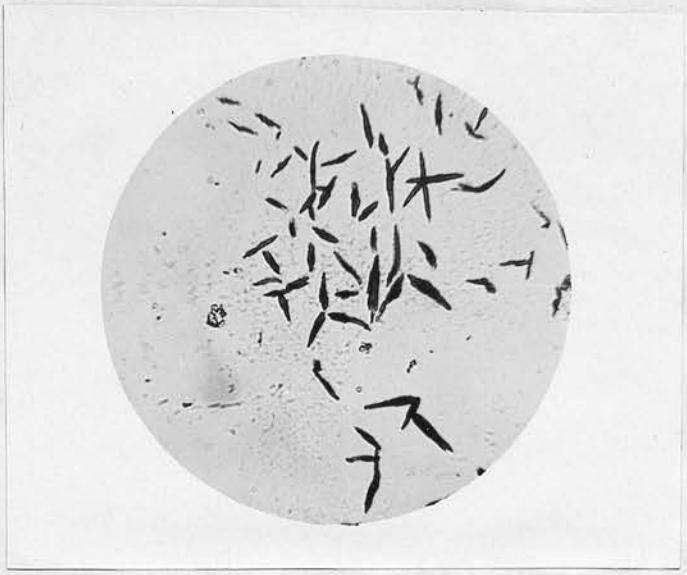


Fig. 5. Indigo crystals (x 450)

Fig. 6. Haemin crystals (x 450)

The crystals obtained with these dilutions are not typical, but much broader and have their corners rounded. There is no doubt that whilst it is not essential that the acetic acid should be glacial, the nearer it is to glacial, the more likely is a successful result. It is stated (Sutherland,Glaister and others) that indigo crystals might be mistaken for those of haemin. With this I do not agree.

Indigo itself is soluble in boiling acetic acid but the method by which garments are now dyed (by bleaching and exposure to air) renders the indigo insoluble, and I have been unable to obtain even a bluish tinge in the acetic acid after boiling with indigo dyed garments. Even if it were possible to obtain a solution from garments dyed in this manner by means of Teichmann's technique, there is no likelihood of a mistake occurring.

Under the low power (Fig 5.) the appearance of a cloud of crystals simulates the appearance produced by haemin crystals, but under a magnification of 350 diameters, the crystals are seen to be of a totally different form to those of haemin (Fig 6.) and to be of a blue colour.

It has also been suggested that crystals of sodium chloride found from salt added to the preparation, might be a cause of error. This seems most improbable as these crystals are colourless.

The successful result of the haemin test chiefly depends on the very careful following of a simple technique .

Many modifications of Teichmann's original test have been proposed, but after careful investigation of these methods, the following is recommended.

METHOD.-

1. Evaporate a drop of normal saline to dryness on a slide. (not necessary in most cases).

2. On the white spot left place a small piece of the stained material, selecting a piece containing a small clot, if possible four or five fibres of the material should be taken so as to ensure a strong localized solution of haemoglobin, crystals may be obtained from much smaller amounts, but with such small amounts the test often fails owing to the blood being all dissolved and becoming too dilute.

3. Place on a cover glass and run in glacial acetic acid.

4. Heat gently until one or two not more, bubbles appear, the acid should not be allowed to boil. Continue cooling and warming, replacing acid lost by evaporation when necessary until with the naked eye the blood colouring matter can be seen starting to diffuse out from the fibres. This may occur almost at once, or only after several heatings. Do not continue heating until the fibres are colourless,or the clot dissolved.

5. Allow to cool, seeing that there is plenty of acetic acid under the cover glass.

6. Examine under the lower power (x75), small crystals will appear in a few moments.

If crystals do not appear, reflood with acetic acid and heat again. Sometimes the haematin is seen gathered in dark masses, in this case the slide should be reheated. If crystals fail to appear, examine again in about ten minutes. Large crystals may be obtained by repeated cooling and heating.

It is important to keep in mind the following points.

1. A SOLUTION OF THE BLOOD MUST BE OBTAINED, AND SUFFICIENT MATERIAL MUST BE TAKEN TO ENSURE A LOCALIZED CONCENTRATED SOLUTION.

2. THE STAIN SHOULD BE DRY.

3. THE ACETIC ACID SHOULD BE AS NEAR GLACIAL AS POSSIBLE.

4. THE HEATING OF THE SLIDE MUST NOT BE EXCESSIVE, THE ACID SHOULD NEVER BOIL.

The crystals appear as dark or light yellow brown rhomboids and rhombs or even diamonds, depending on the mode of preparation and the concentration of the blood solution. The characteristic crystal is the type shewn in(Fig 6)a rhomboid with a nick in either end, though this nick is often not seen. With difficult soluble blood the crystals are shorter and braoder,and owing to ill defined blunted corners may appear oval, the so called whetstone shape.

The crystals are permanent, and may be mounted in Canada balsam for production in court if required.

Bean and Freak (37) arrange a thread to dip into a solution of the suspected material contained in a sublimation tube. After twenty fours hours they examine the preparation, and with a large hand lens are able to see crystals adhering to the upper end of the thread. A considerable time is required to perform this test, and in consequence its value is much diminished, other methods being preferable.

HAEMOCHROMOGEN CRYSTAL TEST. - (Lecha-Marzo Test)

THIS IS A BETTER TEST THAN THE HAEMIN TEST, AND IS ALSO A CONCLUSIVE TEST FOR BLOOD. The test has been known for a considerable time, but owing to difficulties in carrying it out obtained no recognition in medico-legal work.

In 1912 Takayama (39) described two solutions which gave satisfactory results, solution 2 being an improved modification of solution 1, and consisting of

10%	sodium hydroxide	
	Pyridine pure	
Saturated	sol. grape sugar aa	3 cc.
	Aqua destill.	7 cc.

The sodium hydroxide acts as an alkali and as a solvent of the blood. The grape sugar as a reducing agent, and also probably lowers the solubility of the haemochromogen.

Halliburton (12) suggests that the crystals obtained in this manner are a pyridine compound of the haemochromogen, as they are not so easily formed without pyridine, and this is borne out by Anson and Mirsky's work (see page 7). They give the spectrum of haemochromogen.

The solution keeps for one or two months, after which it deteriorates. The yellow colour which develops and which Mahler (40) considers an index of deterioration, is not so, and with a fully saturated solution

HAEMOCHROMOGEN CRYSTALS

Typical appearance when prepared with Takayama's solution

Fig. 7. (x 75)

Fig. 8. (x 450)

Haemochromogen crystals

Fig. 9. Single crystals (x 450)

Fig. 10. Complex formations (x 450)

of glucose it occurs in one or two days.

On the addition of one or two drops of Takayama's solution to a small piece of the suspected material on a slide, in the cold, and covering with a cover glass to exclude air, salmon pink crystals appear in one to six minutes, giving a characteristic appearance under the low power of the microscope. At the same time the colour changes through green brown, dark red, to pink, indicating the formation of haemochromogen, and thus affording confirmation of the test.

If only a minute portion of blood is available this is apt to vanish during any process of heating, a method which can be carried out in the cold therefore possesses a distinct advantage.

Crystals usually appear within one to six minutes when using a solution which has been prepared from 24 to 48 hours or more, but with a freshly prepared solution in the cold, they may take up to thirty minutes to make an appearance. In these circumstances a negative result should not be reported under half an hour. On the other hand, even with freshly prepared Takayama solution, if the slide is heated until bubbles just appear, crystals are formed almost at once. Over-heating has no inhibitory effect.

The crystals themselves are single shallow rhomboids of a salmon pink colour, which when lying on their sides give the appearance of single dark lines, whilst various appearances seen under the microscope, due to

two or more crystals adhering to one another, ~~give~~ give the appearance of sheavea, fir trees and other forms. The individual crystals of these can be made out under higher powers. They may be so large that the spectrum of haemochromogen may be obtained from a single crystal. They may be preserved for weeks or months if mounted in Canada balsam or suitably ringed with a preparation such as gold size to exclude air. Even under the best conditions they tend to disappear.

The test is not interfered with by rust, old age, alkalies, petrol, benzene or putrefaction, and I have never failed to obtain a positive result where blood has been proved present by other means. In the case of stains contaminated by rust, the pink coloured crystals stand out well and are easily seen.

Mahler (40) and Kerr and Mason (36) contrasted the haemin crystal and the haemochromogen test and concluded that the haemochromogen test was the best. The conclusions of the latter were as follows:

A. In the majority of cases, especially with fresh stains there is no difficulty in obtaining haemin crystals.

B. The haemochromogen test however, possesses the following advantages, (1) Simplicity of technique: being performed in the cold by simply adding a solution. (2) There is no danger of overheating, if heat is required. (3) The crystals are easily seen, and have a

characteristic appearance and colour.

(4) The nature of the crystals can be confirmed by the spectroscope.

LECHA- MARZO METHOD.

Balthazard (64) and DOURIS (65) describe this method.

A small portion of the stain is placed on a slide in some drops of pyridine and gently warmed. A drop of an iodine solution in alcohol is then added.

Iodine	2 gr·5	2·5
Potassium iodide	0 gr·5	0·5
Alcohol 95 %	25 grammes (35 cc)	

The preparation is gently evaporated to dryness. A drop of pyridine and a drop of ammonium sulphide are run in under the cover glass. In a few seconds haemochromogen crystals should appear as red brown rhomboids, rectangles or needles.

I have not found this a satisfactory method, and have had difficulty in obtaining crystals. The pyridine and iodine solution do not mix and when obtained the crystals are not as evident as with Takayama's method, to which method the Lecha-Marzo technique cannot be compared.

In addition to these methods other techniques have been advocated, but as they do not bear comparison with Takayama's method, it is not considered of any practical value to discuss them here.

SPECTROSCOPIC TESTS

When light passes through a prism, the violet rays are dispersed to a greater extent than the red rays, and the image found is composed of colours ranging from red, orange, yellow, green to violet.

If the light used is sunlight and a narrow slit is employed, a series of dark lines are visible across the spectrum due to the absorption of these particular rays of light by substances in the atmosphere, the so called Fraunhofer lines. These lines have been named by the letters of the alphabet, and their position is recorded by noting the wave length to which they correspond. A full description of the spectroscope and its principles will be found in any text book on physics.

Haemoglobin and its derivatives each cause absorption bands in the spectrum, and by noting the position of these, the substance can be diagnosed and in some cases its percentage concentration estimated.

To diagnose a substance from a single spectrum with certainty, requires accurate measurements of the position of the absorption bands and even then, two substances may give bands so similar in position, that they cannot be differentiated.

There are numerous substances which give absorption bands similar to those of oxyhaemoglobin, and so the spectrum of oxyhaemoglobin alone is no proof of the presence of blood. We can however, by adding a reducing agent to the solution convert the oxyhaemoglobin into haemoglobin, which shews a different spectrum. We can go further and oxidize the solution, obtaining the original spectrum again, or we may add a weak alkali and obtain the spectrum of haemochromogen and so on.

There is no substance except haemoglobin which will give these different spectra on treating it with these various reagents.

In medico-legal work then, the measurement of the position of the bands is not necessary when the observer is familiar with their approximate position for each derivative; the appearance of the different spectra OBTAINED IN SEQUENCE by the addition of certain known reagents being conclusive proof.

When the accurate position of the absorption bands is to be determined, there are numerous points which may lead to error (Hartridge 60), but where it is only necessary to see the approximate position of the bands, the only source of error is the strength or depth of the solution under examination.

Too strong a solution may cause two bands to appear as one. This is shewn in Fig 11, which gives a graphic representation of the bands of oxyhaemoglobin.

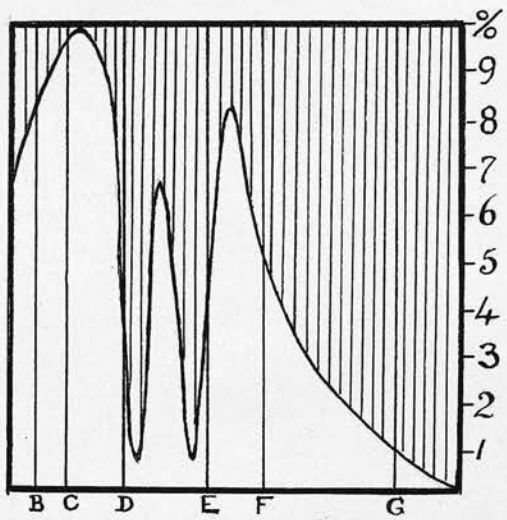


Fig. 11.

A very weak solution may cause one or more bands to be so faint as to be invisible, and a spectrum shewing for instance, four bands, may appear to have only two. This is not likely to cause error except in the case of the inexperienced. Too strong a solution will be readily recognized by the fact that very little of the rest of the spectrum will be seen, and too dilute a solution by the faintness of the other bands and the knowledge of the nature of the derivative under examination.

A suitable concentration is a one per cent solution when used in a test tube, though a 1 in 1990 solution will give readily recognizable spectra if

sufficient depth be employed. Gamgee (63) states the bands of oxyhaemoglobin are perceptible with a dilution of 1 in 100,000, but with the type of spectroscope used in medico-legal work I have been unable to obtain them in such high dilution.

THE MICROSPECTROSCOPE

In addition to being a spectroscope this is a measuring instrument by the aid of which the position of the lines of absorption may be read off directly in wave lengths.

The instrument fits the tube of the microscope after the manner of the ordinary eyepiece, and may be clamped thereon by means of a screw. The upper part of the apparatus which contains the dispersion prism may be swung aside. Looking into the eyepiece in this position of the instrument, a slit will be seen within the lower drum. The microscope should now be focussed on the object. The two milled heads on the slit drum serve for adjusting the length, and width of the slit.

The width of the slit should be narrowed until a pure spectrum is obtained when the top part is swung into position. Using daylight, the Fraunhofer lines will present the appearance of very fine sharply defined hair lines running parallel to the colour bands of the spectrum.

The elbow tube at the side of the upper part of the instrument contains a wave length scale. When the

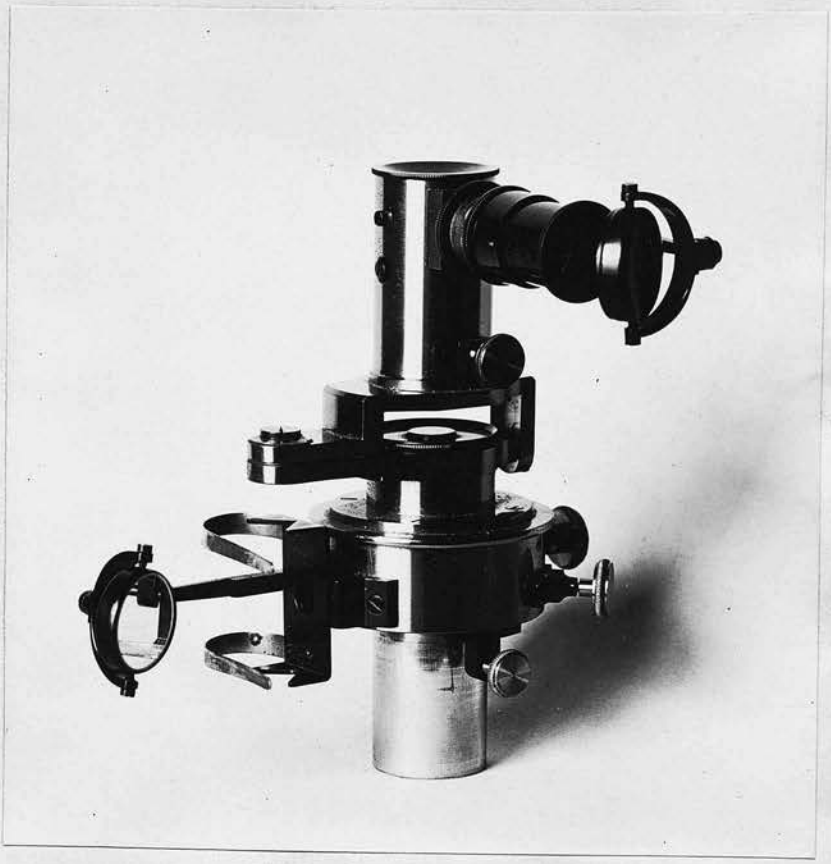


Fig. 12. Microspectroscope.

mirror attached to it is in its proper position the scale will be seen superimposed on the spectrum. The scale may be rendered distinctly visible by manipulating the draw tube of the elbow tube, whilst by slight rotation the scale lines may be set parallel to the Fraunhofer lines.

The body is fitted with a small screw for adjusting the scale. The scale should be set so that the D line (sodium line) in the yellow corresponds to the scale line 58.9 The best methods for obtaining a sodium flame are described below.

The figures on the scale read wave lengths in terms of hundreds of Ångström units that is, 50 represents 5000 units.

A lever at the side of the slit drum serves for putting a comparison prism in and out of action. By means of this, spectra of different substances can be seen side by side and compared.

METHOD OF OBTAINING A SODIUM FLAME.

A suitable flame may be obtained by placing some fused sodium chloride on a piece of gauze under which is placed a bunsen burner. A Fletcher safety burner with the sodium chloride on the grid or contained in a small receptacle also gives a good flame. It is essential that fused sodium chloride be used, otherwise great inconvenience is caused by spurting.

A small bottle containing a mixture of dry sodium carbonate with a little silver sand through which coal gas is passed, will give excellent temporary results, but the bottle requires shaking at intervals. The entrance tube dips below the level of the carbonate, and the exit tube should be made of brass with a small air hole at the base.

ROUTINE METHOD FOR SPECTROSCOPIC EXAMINATION.

Macroscopic Method.

(a) Stain soluble in water.

1. Dissolve in water.

   The solution will give the bands of oxy-haemoglobin or methaemoglobin.

2. Add a reducing agent (Ammonium sulphide, sodium hydrosulphide, hydrazine sulphate)

   The spectrum is replaced by the single broad band of reduced haemoglobin and the solution becomes purplish.

3. Add an alkali to (2) (sodium hydroxide or pyridine)

   The solution becomes cherry red, and the single band of Hb is replaced by the two bands of haemochromogen.

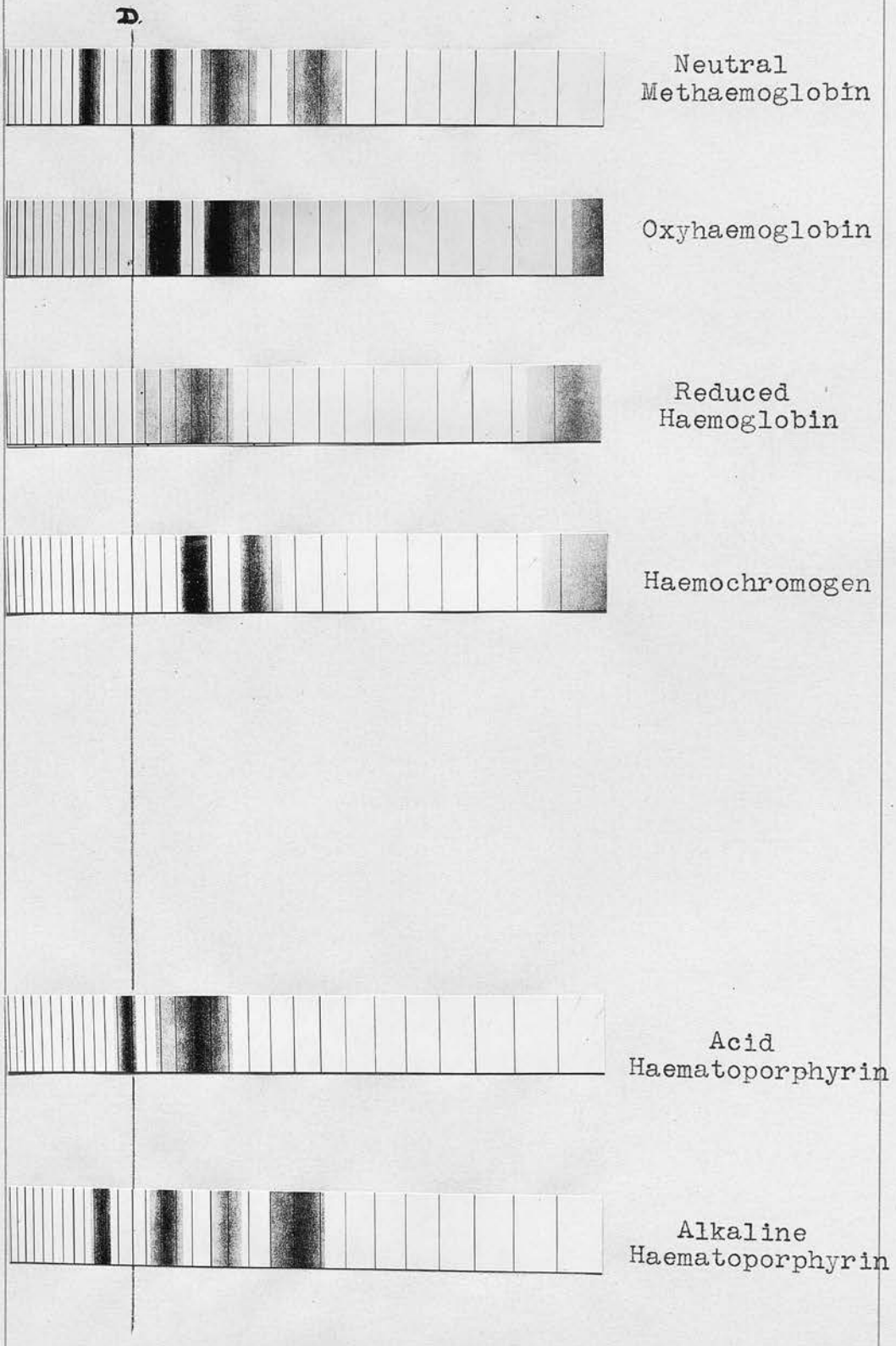
This is sufficient to prove the presence of blood, but if required the process may be continued.

4. Add strong sulphuric acid.

   The solution becomes purplish, and shews the two bands of acid haematoporphyrin, one band being in the red.

5. Make alkaline (see under alkaline haematoporphyrin)

   A reddish solution is now got shewing the four bands of alkaline haematoporphyrin.


D.
Neutral
Methaemoglobin
Oxyhaemoglobin
Reduced
Haemoglobin
Haemochromogen
Acid
Haematoporphyrin
Alkaline
Haematoporphyrin

(b) If the stain is insoluble in water

Dissolve if possible, in sodium hydroxide or dilute hydrochloric acid, make alkaline, and add a reducing agent. The solution will be that of haemochromogen and the examination carried on as above.

If not thus dissolved, remove a small portion, dissolve in strong sulphuric acid and with this solution obtain the spectra of acid and alkaline haematoporphyrin.

The method given above is the most suitable for routine work, but it should be understood that there is no hard and fast rule, and that by a study of the detailed account of the various derivatives of haemoglobin and their spectroscopic appearances given below, many different methods of procedure can be got.

Sutherland writing in Lyon and Waddell (17), recommends dissolving the stain in potassium cyanide and then adding ammonium sulphide, when the spectrum of cyanhaemochromogen is obtained. He considers it a waste of time to obtain any other blood spectra, as blood is the only substance giving this spectrum when treated in this way. With this I do not agree as the infallibilityof the spectroscopic test depends on obtaining a series of spectra, constituting a conclusive test, ruling out any possibility of personal error in estimating the position of the absorption bands.

MICROSCOPIC METHOD.

The same procedure as that just described may be followed, and the test tube placed under the micro-spectroscope. The following method however is very rapid, and particularly useful when only small amounts of stain are present.

1. A small piece of clot or stained material is placed on a glass slide and water run in under the cover glass.

   The spectrum of oxyhaemoglobin or methaemoglobin shews.

2. Without disturbing the cover glass the water is drawn off by blotting paper, and ammonium sulphide or other reducing agent run in.

   The spectrum of haemoglobin now shews.

In a similar way with appropriate reagents, the spectra of haemochromogen and haematoporphyrin may be obtained, all on one piece of blood clot the size of a pin head or less.

If desired, the haemin or haemochromogen crystal test may be inserted after having obtained reduced haemoglobin.

THE MICROSCOPIC DETECTION OF BLOOD

The finding of undoubted blood corpuscles is a conclusive test for blood, but before giving an opinion the corpuscles must be very typical and undistorted. It is only after having looked at a large number of preparations made from dried stains that it is realized how debris of various origins closely resemble slightly distorted blood corpuscles.

In clinical work where fresh blood films are used, there is no difficulty in diagnosis, but whenever a stain dries and is later dissolved again, the corpuscles are distorted, either crenated or swollen and only here and there are typical examples seen.

IT IS ONLY AFTER A MOST CAREFUL EXAMINATION THAT AN OPINION AS TO THE NATURE OF A STAIN SHOULD BE GIVEN FROM THIS TEST, AND EVEN THEN IT SHOULD ALWAYS BE CONFIRMED BY OTHER CONCLUSIVE TESTS IF POSSIBLE.

Its chief value is not as a conclusive test for blood, but rather as a confirmatory test and as a proof of whether a proved blood stain is due to mammalian blood or not.

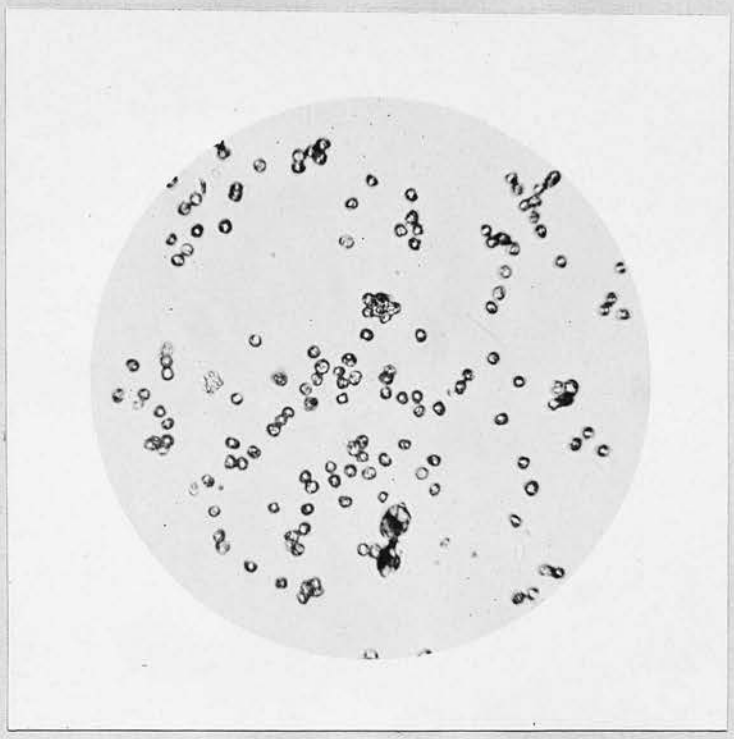


Fig. 13. Microscopical appearance of scrapings from a box sent for examination for the presence of blood. The cells which resemble corpuscles are due to yeast.

THE EXAMINATION OF A STAIN FOR THE PRESENCE OF RED BLOOD CORPUSCLES.

Taylor states that to make it possible to even hope to find recognizable corpuscles, the stain must at the outside, be not more than 24 hours old.

I cannot agree with this. The chief difficulty is to loosen the clot so that the corpuscles may separate out and be clearly visible. In a fresh stain this is quite a simple matter, the blood readily dissolving in a drop of normal saline.

The best procedure is to cut out a strand or two of the stained material, gently teaze this out in normal saline, and examine under a magnification of 250 diameters. If necessary, the preparation should be allowed to macerate for several hours and then reexamined.

In older stains the clot does not dissolve up so readily, and many solutions have been suggested to aid its disintegration.

Any solution used should be as nearly isotonic with the blood as possible so as to avoid distortion of the corpuscles. Normal saline is naturally the ideal fluid, but even concentrations down to 4 % NaCl in water will not distort the corpuscle. In actual practice it will be found that plain distilled water gives perfectly good results in most cases, but if

evidence is to be given based on this test, it is better to use normal saline for obvious reasons.

Of the many solutions suggested as solvents Roussini's fluid is decidedly the best. Mention may also be made of Hoffmann's modification of Pacini's fluid.

Roussini's fluid.-

Concentrated sulphuric acid	1
Glycerine	3
Distilled water to S.G. 1028 at 15 %	

Pacini's fluid (Hoffman).

Glycerine	101 cc
Sodium chloride	2 grammes
Mercurie chloride	1 "
Distilled water	300 cc

Blood corpuscles vary in size, shape, and in possession of a nucleus depending on the species of animal, and whether the blood is mammalian or not.

MAMMALIAN CORPUSCLES are round, non-nucleated bodies, with the exception of those of the camel tribe which are oval and non nucleated.

NON MAMMALIAN CORPUSCLES as in birds and fishes are oval, and possess a nucleus which is quite easily seen.

Under the microscope mammalian corpuscles are are seen to be round, and the centre of the corpuscle will appear lighter or darker than the periphery owing to the biconcave nature of the corpuscle, and depending on the light employed.

By altering the amount of light passing through the microscope or by altering the focus, the appearance of the centre of the corpuscle can be changed from light to dark, as contrasted with the periphery, or vice versa. This difference between the centre and the periphery must not be mistaken for a nucleus.

MEASUREMENT OF CORPUSCLES.-

At one time Schmidt (1848) and others attempted to diagnose the species of animal from which the blood originated, by measuring the diameter of the corpuscles and even now, some well known text books still toy with the idea in modified form.

The variations in the size of blood corpuscles in normal human blood is such that in the range covered by the variation, some of the domestic animals are overlapped, and not only that, but the actual treatment the corpuscles undergo, such as drying and moistening before they are measured, also alters their size, and lastly, in blood diseases and many other conditions the variation in size is even greater than in normal blood.

The following is the average size of the corpuscles of some of the more common domestic animals.

The average results of different observers are given.

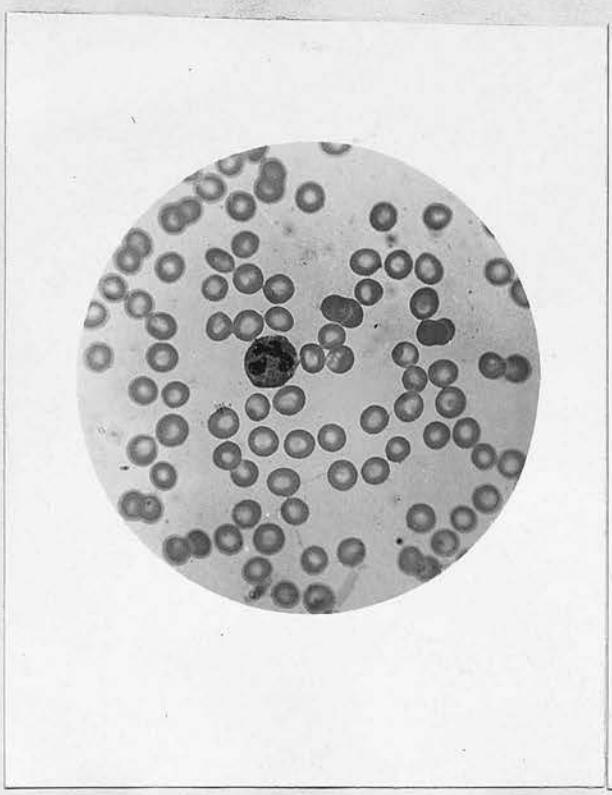
Human	·0079 mm - ·997	about 1/3200 of an inch
Dog	·0074 mm -·0066	
Rabbit	·007 mm - ·006	
Ox	·0062 mm -·0054	
Pig	·0065 mm -·0046	
Horse	·006 mm -·0053	

From the above it will be seen that the average size of fresh healthy human blood corpuscles is slightly larger than those of domestic animals.

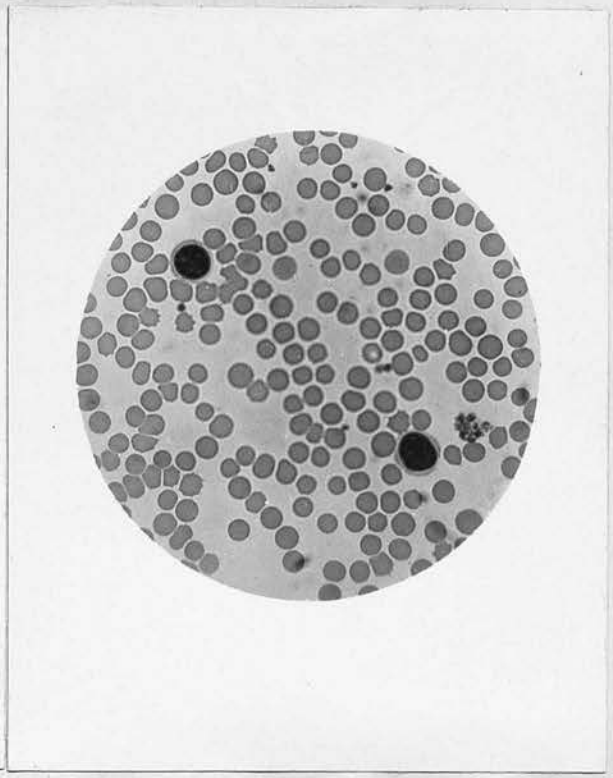
In preparations made from stains, the corpuscles have subjected to conditions which alter their size ~~an~~ and shape, and even in recent stains the number of corpuscles, sufficiently normal to measure is very limited, and not numerically sufficient to give an accurate average, though in films taken direct from healthy blood a good idea may be obtained of the origin of the blood.

In medico-legal work NO OPINION REGARDING THE ORIGIN OF THE BLOOD CORPUSCLES, FOUNDED ON THE MEASUREMENT OF BLOOD CORPUSCLES SHOULD EVER BE EXPRESSED IN A COURT OF LAW. AT THE MOST, ALL THAT CAN BE STATED IS THAT THE SIZE OF THE CORPUSCLES ARE COMPATIBLE WITH THE BLOOD BEING OF SUCH AND SUCH AN ORIGIN.

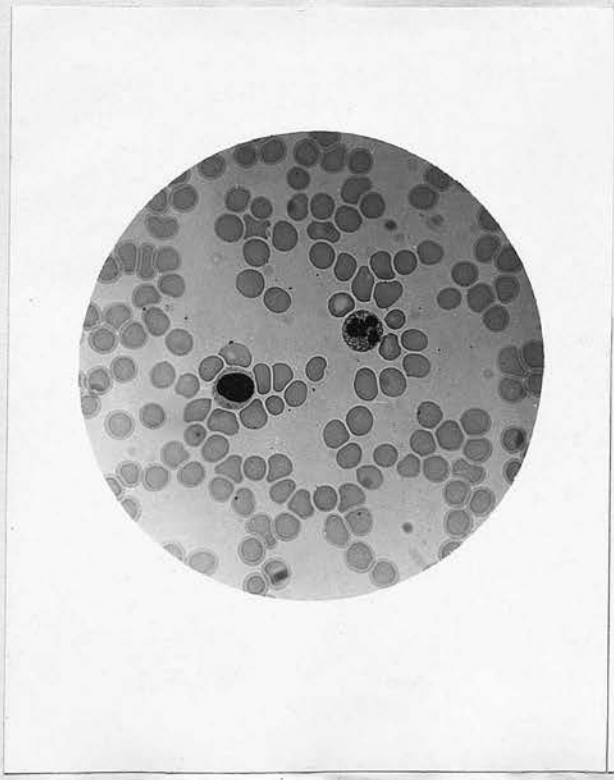
BLOOD CORPUSCLES (x500)



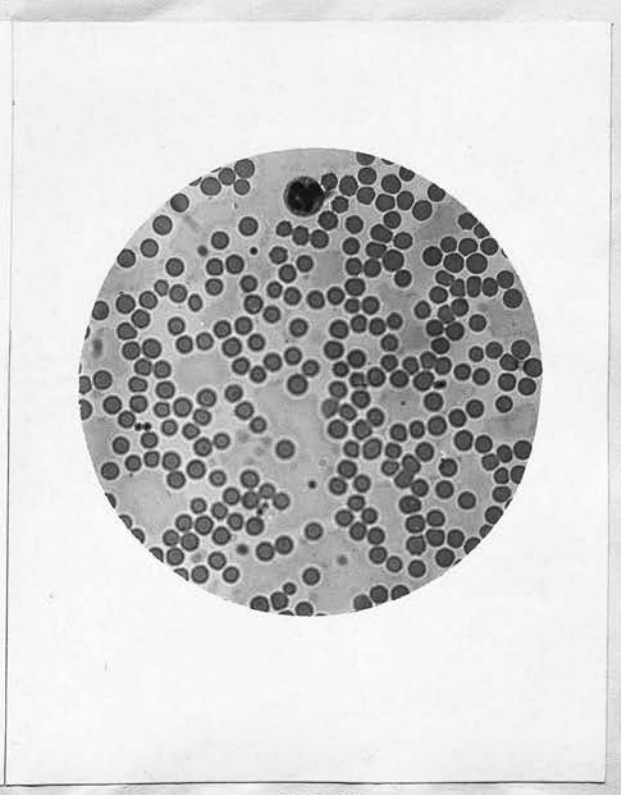
HUMAN



OX



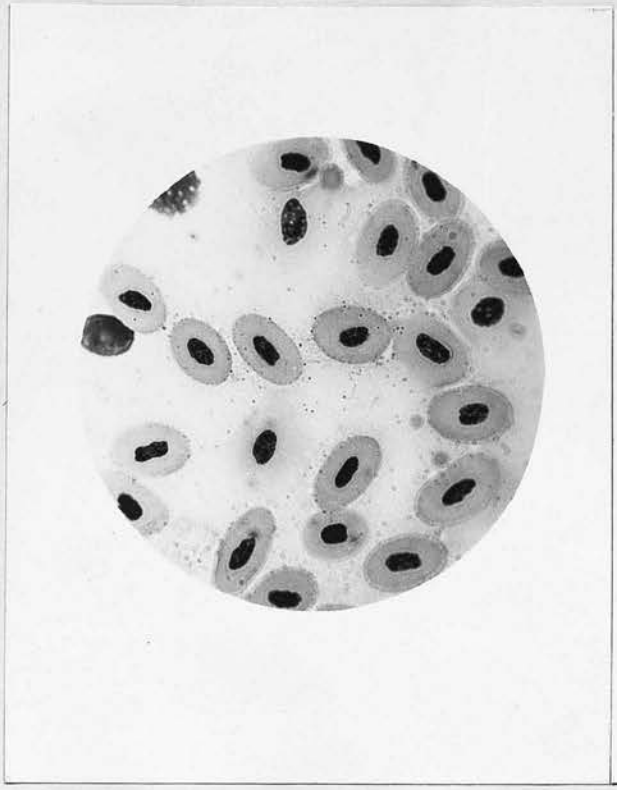
RABBIT



SHEEP

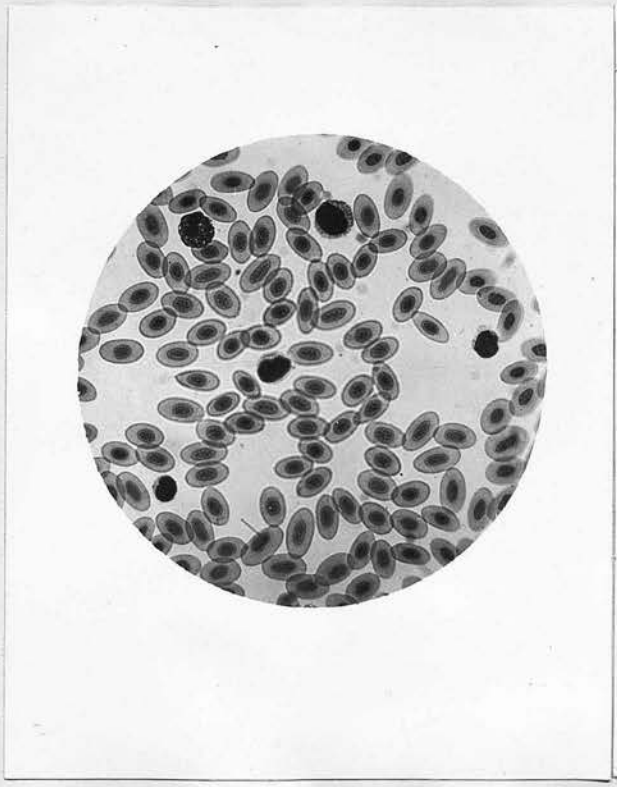
FIG. 14.

BLOOD CORPUSCLES (500)

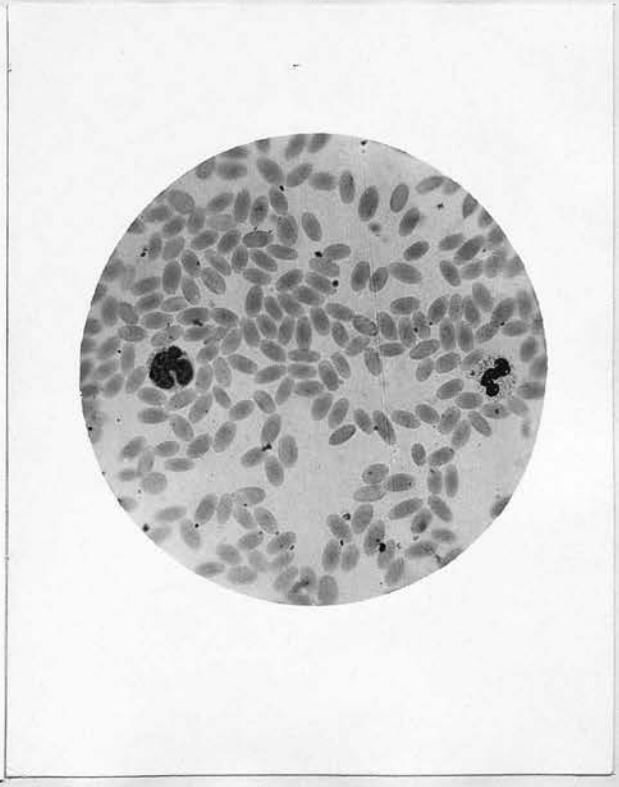


FROG

FISH



HEN



CAMEL

FIG. 15.

METHOD OF MEASURING AN OBJECT UNDER THE MICROSCOPE

For this purpose some kind of micrometer eyepiece is required. This may be an ordinary ocular fitted with a flat piece of plain glass on which has been ruled a scale divided into tenths and hundredths

The divisions do not represent any definite measurement and the scale requires to be graduated for each combination of objective and ocular and for the particular length of drawtube in use. This is done by means of a STAGE MICROMETER, which consists of a glass slide on which are ruled parallel lines. The distances between the lines are known, and usually represent one tenth and one hundredth part of a millimetre, sometimes one hundredth and one thousandth of an inch. One thousandth of a millimetre is the unit called μ . The scale is generally about a millimetre in length.

To graduate the eyepiece scale, the eyepiece is first focussed by manipulation of the drawtube to the eyepiece itself, until the markings of the scale are clearly seen.

The rulings on the stage micrometer are then focussed giving the appearance shewn in Fig 17, where the images of the two scales are seen superimposed. The markings on the stage micrometer are more easily seen with the iris diaphragm almost closed.

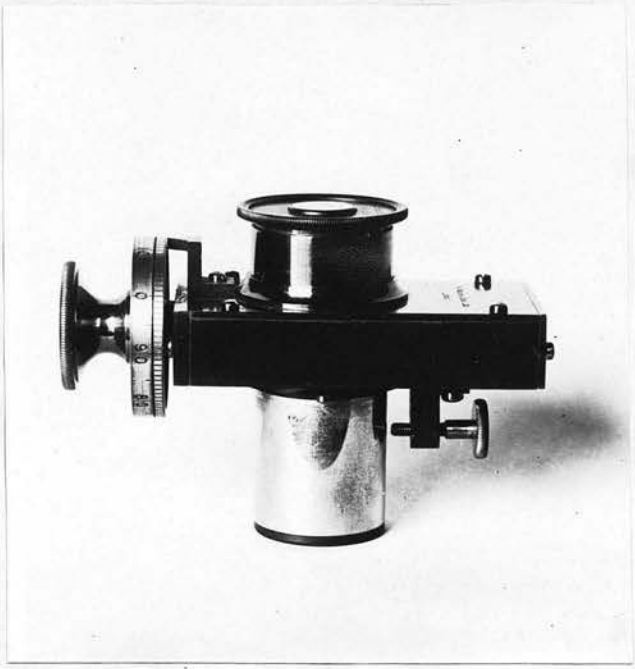


Fig. 16. Eyepiece micrometer.

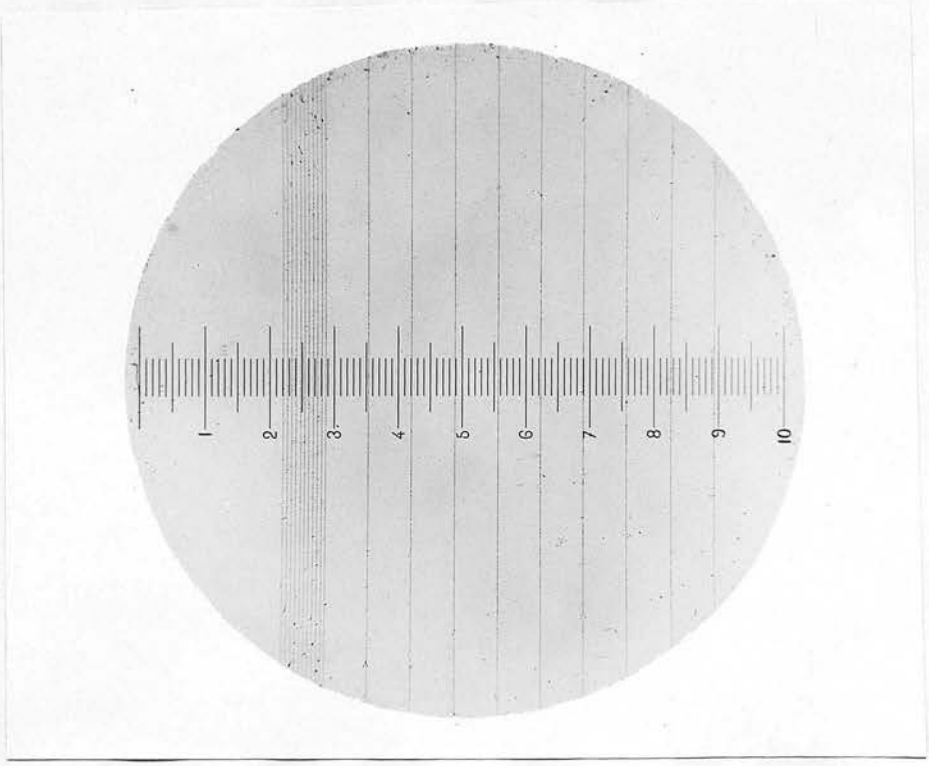


Fig. 17. Stage micrometer, with eyepiece scale superimposed.

The number of tenths of a millimetre that accurately correspond to a definite number of graduations on the eyepiece micrometer are noted, and the distance between each division on the eyepiece scale calculated. For instance, if two large divisions (twenty small) on the eyepiece scale correspond to one of the divisions on the stage micrometer which is known to be one tenth of a millimetre, then each large division of the eyepiece scale corresponds to one twentieth of a millimetre.

Instead of the eyepiece micrometer with a fixed scale, an eyepiece with a movable hairline may be used, (Fig. 16). In this instrument the field shews one fixed line, and one movable line; the latter is moved by means of a micrometer screw graduated in tenths of a millimetre, shewn at the side of the instrument. For rough purposes, the size of the object may be obtained by dividing the reading on the micrometer scale by the initial magnifying power of the objective, but for accurate readings, the scale must be graduated before use by means of the stage micrometer slide to determine the exact value of the graduations on the scale.

CRYSTALS OF OXYHAEMOGLOBIN AND HAEMOGLOBIN.

Attempts have been made to identify the species of animal to which a given blood belongs by means of the shape of its haemoglobin crystals.

Copeman (51) and many others have made a study of the subject, and Reichart and Brown (52) prepared oxyhaemoglobin crystals from the blood of ever one hundred species of animals and studied their characteristics.

Oxyhaemoglobin may crystallize out in many different forms such as prisms, plates, tetrahedra or needles depending on the animal. A different form of crystal is obtained from each species.

Crystals are obtained with great ease from some animals such as the horse, dog, squirrel, guinea pig, and rat. In these cases the crystals are sparingly soluble in water.

Crystals from bullock, sheep, pig, and human species are very soluble in water, and so only obtained with difficulty.

Glaister (53) states that the crystals obtained from human blood are those of reduced haemoglobin, whereas the crystals from the lower animals, are of oxyhaemoglobin, and that after research this may be of value. I have not verified this point.

The crystals of haemoglobin are more soluble than those of oxyhaemoglobin, more difficult to obtain,and are as a rule isomorphous with the corresponding oxy-haemoglobin crystals, but of a dark purplish colour.

The preparation of crystals of human blood, which is the type of blood most likely to be met , is not easy, and when prepared it requires an expert crystal-lographer to interpret the results.

The precipitin test which has been introduced since Reichart and Brown (52)commenced their extensive investigations, is reliable in the hands of an expert and not fraught with such difficulties. On this account alone the question of advisibility of attempting to determine the origin of blood in medico-legal cases by means of crystallography is permanently settled.

Crystals are easily prepared from rat's blood, by mixture with a drop of ether, and allowing evaporation on a slide. Other bloods require more elaborate treat-ment. A summary of the many methods described will be found in Reichart and Brown. They need not be described as the subject has not a practical medico-legal value.

DIFFERENTIATION BY SPECIFIC HAEMOGLOBIN

Recent work by Barcroft on the blood of the worm Arenicola has shewn quite definitely that both in its physical and chemical properties, that animal at any rate, possesses its own specific haemoglobin.

Barcroft's work was carried out with the Hartridge reversion spectroscope (q.v.). Using this instrument, Barcroft found that the α band of haemoglobin from Arenicola is in quite a different place from that of human haemoglobin. As this instrument will be increasingly used in forensic laboratories it may be that after further investigations it will be practicable to differentiate the origin of blood by this method.

THE AGGLUTININ TEST FOR HUMAN BLOOD.

This test depended on a mixture of blood with the blood serum of another animal of different species shewing agglutination of the corpuscles.

The present knowledge of blood groups shews that it is not reliable, as certain types of human blood will agglutinate with other types of human blood.

If it is proposed to use the test, care must be taken that the known human blood used for the test is of group I (see 'blood groups'), as this group will not agglutinate with other types of human blood. The test is not now used, but is entered here for the sake of completeness.

THE ANAPHYLAXIS TEST FOR HUMAN BLOOD.

I have no experience of this test which is never used but is here included lest it be rediscovered in the future.

The test is based on the fact that when an animal receives an injection of a foreign albumin, it develops hypersensitiveness to further injections of this particular albumin. A further minute injection causing a rise of temperature. The most useful animal is the guinea pig, from its extreme sensitiveness.

If, for example, 1/100cc of sheep's blood is injected into a guinea pig, and 14 days afterwards a minute dose of 1/200,000 cc be injected, the body temperature will rise for about one hour, whereas a previously untreated guinea pig will require possibly 1/20cc sheep's serum to cause its temperature to rise. With larger doses an untreated guinea pig's temperature will fall after a dose of 1 cc serum whilst a treated one will only require 1/10,000 cc or less to affect it.

By having a series of guinea pigs sensitized with serum from various animals, injecting them with a portion of the suspected stain extract, and recording the rectal temperature the origin if the stain may be determined. The guinea pig shewing marked rise in temperaturehaving been sensitized with blood serum of similar origin to the suspected stain.

PRECIPITIN TEST

The result of injecting the serum of almost any animal into a rabbit, is to produce in the blood of the rabbit, a body which brings about the formation of a precipitate when the latter's serum is mixed into the blood of the former, the serum of the treated animal is spoken of as an anti serum to the species of animal from which the injected blood was derived. Thus, if a rabbit is immunized with human blood, we obtain an anti human rabbit's serum.

The reaction is specific in high dilutions. With concentrated solutions of blood serum a group reaction occurs, and the anti serum will give a precipitate with the blood of all animals of that group, (i,e,with all mammals, the so called mammaliam reaction) On this account a dilution of not less than 1 : 1000 blood serum must be used. Time is also an important factor. Provided sufficient time is allowed to elapse, the blood of other mammals will give a precipitate with human anti serum or vice versa. For forensic work, a cloudiness should appear in from two to five minutes at room temperature for a positive result.

The use of high dilutions of stain extract disposes of the group reactions. There still remains a difficulty in regard to closely allied species such as rabbits and hares, horse and mule, man and apes,etc the method of dilution is unsatisfactory in these cases.

Uhlenhuth introduced what he called cross immunization to eliminate the similarity between simian and human blood, or between other allied species should such a question arise. Relying on the fact that an animal will not produce precipitins against blood of its own species, he inoculated monkeys with human blood serum and obtained an anti human simian serum which precipitated human, but not simian blood. He also obtained an anti hare rabbit serum which precipitated hare's blood solution, but not rabbits.

The precipitin reaction is not a test for blood but for the origin of the protein contained in the blood. The test will be given by any protein derived from the same animal as the blood which gives a positive test. It is essential therefore, that the substance being tested must be proved to be blood by other means. The precipitin test cannot even be used as a confirmatory test. The practice of some examiners of testing the suspected stain by the guaiac test (q.v) and then submitting it straight away to the precipitin test, and forming an opinion on the results obtained from these tests, cannot be too strongly condemned.

The stain extract.

The first step is to obtain a clear solution of the stain. This is done by soaking a small portion of the stained material in normal saline, if the stain is on metal or wood, a small portion should be scraped off and dissolved in saline. As a control an unstained portion of the clothing should be treated in a similar manner to shew that it alone does not give the reaction.

Sufficiently strong solutions can be obtained in a few minutes with some stains; with old stains however, prolonged soaking may be necessary, such stains should be allowed to stand overnight. Prolonged soaking is best done in a cold place, or refrigerator, in order to avoid any likelihood of bacterial growth although blood which has been putrefying for several months will give the reaction.

The solution obtained should be cleared by filtration or centrifugation if necessary, and should be neutral to bitmus, if not neutral it should be made so by the addition of tartaric acid or sodium bicarbonate as acids and alkalis may cause a false reaction. The extract for use should not be stronger than 1 : 1000. The strength may be estimated by gently shaking the fluid and diluting until a persistent froth just remains upon the surface. This indicates a strength of about 1 in 1000. A second ~~mentioned~~ method is to boil some

of the extract and add a drop of 25 per cent HNO_3, a faint opalescence indicates a strength of about 1:1000 If a heavy precipitate forms the extract must be further diluted with saline.

The anti serum

This must be highly potent and absolutely clear. After long standing, a precipitate may be deposited in the tube. The clear serum should be carefully drawn off and is then quite fit for use. If the anti serum has become cloudy, it may be possible to clear it by filtration. Kolmer, McWeeney, Graham Smith and others state that even an infected and offensive serum will give the reaction, and I have found this to be the case. Forensic precipitin anti sera are now manufactured commercially by Burroughs and Welcome and I have found them to be very satisfactory.

Graham Smith and Sanger (96) found that intravenous injections into a rabbit of 5,5,5, and 3 cc of human serum at intervals of 2,3,and 4 days produced a powerful anti serum. The animal was bled 14 days after the last injection.

The test.

This is best performed in a series of small test tubes. A small amount of anti serum is first placed in the bottom of the tube and the stain extract then run in carefully so as to layer over the fluid already

present, a positive result is indicated by a ring at the junction of the two liquids. (~~355~~ 399 photo). This ring must be compared with the control, since a slight opalescence is sometimes seen at the line of junction of salt solution and serum, not due to precipitation.

In the event of only a small amount of extract being available, the test may be performed by drawing up some of the extract into a capillary tube and underlying this with anti serum, when in a suitable light, a ring of precipitation can be seen at the junction of the fluids. The tubes are best viewed against the shadow cast by the working table, no light coming from the side or behind (Colles).

I have obtained satisfactory results with this method, but consider the use of small test tubes very much better, less liable to error, and a method which should be used for preference in any case where there is the slightest doubt about the result.

Proportion of anti sera to amount of stain extracts.

The precipitate produced by the interaction of anti sera and its antigen is soluble in excess of either anti serum or antigen. Dean and Weiss (77) have shewn that there is an optimum ratio between the amount of antigen and antiserum, and dilution of the extract. For practical purposes the range is very wide and 1 part of potent anti serum to 10 or 20 parts of a 1:1000 dilution of stain extract will give satisfactory results.

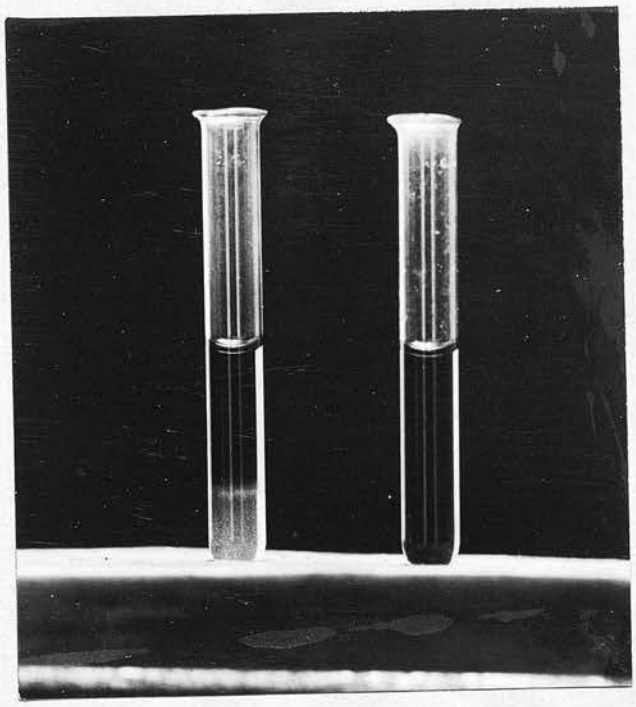


Fig. 18. Precipitin Test.

Positive (left) and negative results.

Technique of test.

There are numerous modifications in technique, all equally good. In the case of blood suspected to be human, Kolmer (78). uses the following.

Tube 1. .1 cc Anti human serum + 2 cc unknown extract 1:1000

2. .1 cc anti human serum + 2 cc unknown extract 1:5000

3. .1 cc - - - + 2 cc - - 1:10,000

4. .1 cc normal rabbit serum + 2 cc - - 1:100

5. .1 cc anti human serum + 2 cc human serum 1:1000 (anti serum control)

6. .1 cc - - - + 2 cc normal saline (saline control)

7. .1 cc - - - + 2 cc extract of unstained portion of garment (control)

The first three tubes should shew a cloud in from 2 to 5 minutes. I prefer to control the anti serum with serum immune to some other animal and proceed as follows.

Approximate amounts

Tube 1. .1 cc anti human serum + 1 cc unknown extract 1:1000

.. 2. .1 cc - - - + 1 cc sheep's serum 1:1000

.. 3. .1 cc - - - + 1 cc human serum 1:1000

.. 4. .1 cc anti sheep serum + 1 cc unknown extract 1:1000

.. 5. .1 cc - - - + 1 cc sheep serum 1:1000

.. 6. .1 cc - - - + 1 cc human serum 1:1000

.. 7. .1 cc anti human serum + 1 cc extract unstained (portion of clothes)

The human and sheep's blood I obtain from handkerchiefs which have been dipped in the respective blood and allowed to dry, in this way various bloods are always

at hand in the Laboratory for extractions and comparison and the resulting extract has the merit of having undergone the same treatment as the unknown stain.

Tube 3 and 5 acts as the anti serum controls,

Tube 2 and 6 acts as the saline control. Should tubes 1 and 4 both give a positive reaction within five minutes, the probability is that the unknown extract is too strong, and the test should be repeated with greater dilutions. These tubes thus give a control of the concentration of the unknown extract. The possibility of both bloods being present must however be borne in mind especially if the accused alleges the blood is that particular animal whose anti sera is used in tube 4. In such a case a third antiserum must be used in the test, and the extracts of human, sheep, unknown serum and its own specific blood tested against it, as well as testing the dilutions of its own specific blood used, against the anti sera used in the original test.

A third cause for the reaction in both tubes may be the presence of unneutralized acid in the stain extract. Cloudiness appearing after five minutes is of no importance and must be ignored, as it is not specific when employing serums of the potency used in forensic work.

Fallacies

The chief fallacies are the use of too concentrated stain extract. The precipitate which forms on standing being taken as a positive result. The possibility of free acid in the stain extract causing a precipitate and the fact that other animal proteins and secretions from blood will give a positive reaction.

These questions as well as the difficulty of distinguishing between the blood of closely allied species have already been discussed.

The nature of the material stained is stated to affect the test; particularly stains on leather, the substances used in the tanning being stated to produce a positive reaction. I have not come across any case of this nature. The reactions may be inhibited by contamination by various substances and care should be taken to see that the stain extract is neutral

Graham Smith found that formalin, corrosive sublimate and iron and copper sulphate destroyed the precipitate forming power of the blood, whilst lysol and similar antiseptics formed such clouding with salt solution as to render the test of doubtful value in their presence. He found that age, drying and putrefaction did not destroy the capacity of the blood to act, and with this I agree. I have also found that some sera will cause a cloudiness with any blood however dilute. This was described by Nuttall (79), who referred to the serum as opalescent serum.

Summary

The precipitin test is a reliable test for the origin of a blood stain, but should only be used by persons having experience with the test. It is the usual test employed in forensic work and has the advantage over the complement fixation (q.v) test, in that there are fewer manipulations and so less likelihood of error. It has the additional advantage that the only reagents required are the various anti sera.

Workers in bacteriological laboratories may however, prefer the complement fixations test.

SPECIFIC HAEMOGLOBIN PRECIPITINS IN DIFFERENTIATION OF BLOOD.

As already stated the precipitin test is not a test for blood and the presence of blood must be proved by other means. Hektoen & Schulhof (80) prepared precipitin serums for haemoglobins which have the advantage over the ordinary precipitin serum which only prove the presence of albumen in general, in that they prove the presence of haemoglobin and so of blood.

To obtain a haemoglobin precipitating serum they use a suspension of red blood corpuscles. This has been freed through repeated washings, from every trace of serum, five or six intravenous injections at intervals of three days, provide after 4 or 5 days a specific serum for haemoglobin, which does not affect serum.

Precipitating anti sera obtained with suspensions of corpuscles or pure haemoglobin are as specific and sensitive as the usual precipitating serums obtained by injection of serum.

COMPLEMENT FIXATION TEST.

If a rabbit is injected with human blood serum (antigen) under suitable conditions, its serum will develop antibodies which interact with human blood.

This interaction depends on two components in the rabbit's serum, the antibodies artificially produced and a substance called complement normally present in the blood serum of all animals. Such a serum is known as, an immune anti human serum.

If human serum is brought into contact with immune anti serum which contains complement, an interaction takes place, although no visible change is produced, which results in complement being used up or fixed. If however, the immune anti serum is heated to 55° ~~per~~ Cent. for half an hour, the complement is destroyed and no interaction takes place until complement is added. If absent from the human serum, as is the case when performing the test for blood, it may be added in the form of a small amount of fresh guinea pig serum.

We have then,

A. Human serum or human blood stain extract (antigen) in which the complement has perished.

B. Rabbit's serum containing anti human antibodies but no complement, this having been destroyed by heat.

C. Fresh guinea pig serum containing complement.

If these three are brought together interaction takes place, and by estimating the strength of each component and adjusting the amounts of each present, it can be arranged that the whole of the complement is used up or, as it is called "fixed".

Should serum or stain extract from another animal be substituted in place of human serum as the antigen then no interaction will take place and the complement will not be fixed. That is, there will be free complement available.

All that is necessary then to ascertain whether serum'A'is human or not in the present case, is to find out if there is free complement present, after the three sera have been brought together under suitable conditions (incubation and dosage). In the same way by using the necessary antiserum, the presence of the blood of any other particular animal can be tested for.

To ascertain whether free complement is present.

A rabbit is immunized against sheep's corpuscles so that its serum develops antibodies which haemolyse sheep's corpuscles in the presence of complement. This anti sheep haemolysing serum is heated to destroy its complement leaving only the antibodies (amboceptors).

This serum will not now haemolyse washed sheep's ~~or~~ corpuscles until complement is present, and the two together (amboceptor and sheep's corpuscles) compose

an indicator of the presence of complement. If the result of the main test (stain extract and antiserum + complement) is to leave free complement present, then on adding the indicator, haemolysis of the sheep's corpuscles will take place; if the serum being tested (antigen) was due to human blood, then the complement will have been fixed, no free complement will be present, and so there will be no haemolysis of the sheeps corpuscles.

In actual practice the test has many modifications and is open to many sources of eror. It should only be attempted by one thoroughly conversant with complement deviation work of all kinds. The test takes a ~~vey~~ very considerable time to carry out apart from the titration of the various components. For workers in a bacteriological laboratory where complement fixation work is frequently carried out, the test may recommend itself, for ordinary forensic laboratory work however, it cannot compare with the precipitin test for simplicity and rapidity, and it is not now used in actual forensic practice.

It has been urged that the complement fixation test gives a qualitative result and that on that account has an advantage over the precipitin test. In some branches of the subject, that is so, but in forensic work this is not the case, as the problem to be determined is whether the test is positive or negative. The question of degree does not matter and is of of no value or interest.

It is beyond our range to discuss possible fallacies and precautions necessary in performing this test. These can only be realized by a long study of the subject. Each worker will use quantities which his experience tells him will be approximately right. The following scheme which I have used may serve as a rough guide.

Row	Antigen 0.5 cc dilution depends	Antisera 0.05 cc 1 part in	Complement 1 in 10 units											
								antigen control				anti-sera control	
			2	4	8	12	18	2	4	6	8	2	4
1	on anti-comple-ment titrate	10											
2		100											
3		1,000											
4		10,000											
5		100,000											
Tubes			1	2	3	4	5	6	7	8	9	10	11

Incubate for 1¼ hour at 37°C; add 0.5cc sensitized corpuscles to each tube; incubate for ½ to 1 hour at 37°C.

BLOOD GROUPS

The examination from a living person, a corpse or a stain by means of isoagglutinations may be of considerable value in some cases.

The determination of whether a blood is human or not by this means has already been considered (p 74).

This examination may further be of use in cases of legitimacy, substitution of children, paternity etc, and in cases of assault or murder where blood on an accused persons clothes, though proved to be human blood may however, be proved not to be that of the deceased or not that of the accused. It may also in some cases answer the question of whether stains found on garments in cases of sexual assaults are due to menstruation or not.

We have now reached a stage when conclusions drawn from the examination of fresh blood from a living person may be accepted as accurate and reliable for forensic purposes. The question of the examination of dried stains has also been investigated thoroughly, though so recently that it has not yet stood the test of time. The examination moreover, needs considerable experience. The field is a large one.

I have not personally confirmed the observations of Schiff (96), Lattes (97), Reinheimer (98), Snyder (99), Bernstein (100), Strassman (101) and many others. Their work to which I shall refer, has however, been

comfirmed by other observers and I intend here to summarize the present position of this examination of blood. The works quoted above furnish full references on the subject.

CLASSIFICATION OF HUMAN BLOOD.

According to the theory of Landsteiner, human blood contains bodies which cause agglutination of the red blood corpuscles, when it is mixed with blood serum from certain other individuals. As it is the blood corpuscles which are agglutinated, we speak of haemoagglutination, and as it is caused by the serum of the same species ; Isohaemoagglutination.

Landsteiner's theory has now been proved to be inaccurate in details, and modifications have taken place; as however the principle is the same, a clear description may be given of the present position by a consideration of his theory. It is adopted here, the additional modifications being mentioned later. These modifications only affect the inheritance of the bodies, and for other purposes Landsteiner's theory still holds good.

The bodies concerned are not developed like the agglutinins of bacteriology by immunizing, but are existing factors in normal serum. They consist of two bodies in the corpuscles, referred to as A and B agglutinogens, and antagonistic bodies in the serum distinguished as α and β agglutinins.

According to the presence or absence of two or more of these bodies, it is found that the blood of human beings falls into four groups. On placing two

similar bloods together no agglutination takes place, if however, they are dissimilar there may be aggluti - nations depending to the relative groups to which the bloods belong. By testing an unknown blood with samples of blood from known groups, it can be determined which bodies are present in the unknown blood, and so to which group it belongs.

Nomenclature

According to the original classification of Jansky, the groups were called I.II.III.IV. Moss however, grouped blood so that his group I corresponded to Jansky's group IV and vice versa. Groups II and III remaining the same.

It is safer as has been urged by many observers, to allude to the group by the letters of its contained bodies. No mistake can then occur. If absence of these bodies is indicated by O, we have

Jansky Group	Agglutinogen in Corpuscles	Agglutinin in Serum	Formula
I	O	Anti A or α Anti B or β	O$\alpha\beta$
II	A	Anti B or β	Aβ
III	B	Anti A or α	Bα
IV	AB	Nil	ABo

Reference to the above table shews that if the corpuscles of group II (A β) are brought into contact with the serum of group III (B α) they agglutinate; if however group II (A β) is substituted for group III no agglutination occurs.

Bringing the unknown blood into contact with known serum of group II (A β) and group III (B α) and noting the presence or absence of agglutination, grouping of the unknown blood can be determined, as shewn by the following table.

Unknown blood group	Known serum group II(A β)	Known serum group III(B α)
I(O $\alpha\beta$)	—	—
II(A β)	—	+
III(B α)	+	—
IV(AB o)	+	+

It should be noted that the bodies A and B, although in the formulae of the groups II and III are contained in the corpuscles and so are not present in the test sera.

In the same way unknown serum may be tested against known corpuscles A and B.,

Unknown serum group	Corpuscles group II(A β)	Corpuscles groupIII(B α)
I(O $\alpha\beta$)	—	—
II(A β)	—	+
III(B α)	+	—
IV(AB o)	+	+

Wherever possible, as for instance in cases of paternity, it is better to use both of these methods, one acting as a control to the other.

Providing that blood of known group II (A β) or of group III (B α) is available, the single known blood will determine the group of the unknown blood if the above methods are combined

Serum and corpuscles of group II used to determine grouping of an unknown blood

Known serum group II(A β) and unknown corpuscles	Known corpuscles group II(A β) and unknown serum	Unknown group
—	+	I (O $\alpha\beta$)
—	—	II (A β)
+	+	III (B α)
+	—	IV (AB o)

Serum and corpuscles of group III used to determine grouping of an unknown blood

Known serum group III(B α) and unknown corpuscles	Known corpuscles group III(B α) and unknown serum	Unknown group
−	+	I (O $\alpha\beta$)
+	+	II (A β)
−	−	III (B α)
+	−	IV (AB o)

Remembering that blood causes an agglutination if brought into contact with blood of another species, and that this can be used as a test for human blood, it is necessary, before determining the blood groups, to eliminate this source of agglutination, by first proving that the sample to be tested is blood, and also that it is human.

Constancy of blood groups.

The bodies A and B in the corpuscles are always present at birth, but not so the agglutinins α and β. these are developed in the course of the first year, and after this the blood group does not change. A few cases described with change in an individual's blood group being simply due to faulty technique.

Thomsen (102) has described a change in the group of a blood occurring in vitro after standing 48 hours, this he ascribes to a latent receptor L, which is covered under physiological conditions. Fresh blood from the patient was always of the original group.

Heredity of blood groupings.

The inheritance of blood groups follows Mendel's law. According to the older theory there were two independent factors A and B which were dominant to absence of A and B.

The importance of heredity in blood groups is in the case of disputed parentage, and using the older theory no serious error will be made, because no conclusive statement to the effect that it is not possible for the parents to be parent and child, will be made. The possible error being that an observer may conclude that parentage is possible, when it is not. This is

a generalization so wide that its definite value as evidence is nil.

Bernstein (100) has described a new theory which fits the existing facts better and places tables such as that of Roche (103), which shew the results of unions of different groups, out of date. He shews that the relative proportions of the various groups in the population correspond to what would be expected from his three allelomorph theory, but vary materially from what would be expected from the older so called dihybrid theory. Bernstein's theory does not invalidate previous tests but makes their application more close. It limits the groups of the remaining parent to a smaller number of possibilities in cases in which the other parent is in group IV; only in the case of a parent of group IV is any change made. He states that the blood groups are inherited not as two independent pairs of factors, but as three multiple allelomorphs. That means that the genetic factors responsible for the four groups are all located at the same spot on a single pair of chromosomes. He considers the basis of the four groups to be three factors, a recessive R, and two dominants A and B. Thus, group I ($\alpha\beta$) has the genetic formula RR.

Group II ($A\beta$) = AA or AR, Group III ($B\alpha$) = BB or BR and Group IV = AB. On this hypothesis it is easy to see that a Group IV (AB) parent, can never have a child of Group I (RR). Group IV containing two dominants. Union of Group I (RR) and Group IV (AA) can only result in offspring of Groups II (AR) and III (BR), instead of all four groups as was formerly thought. The tables shew the groups of children the parents being known, and the remaining parent when the child and one parent are known.

Table shewing blood groups of children, the parents being known.

One parent group.	One parent group.	Children groups.
I	I	I
I	II	I.II
I	III	I.III
I	IV	II.III
II	II	I.II
II	III	I.II.III.IV
II	IV	II.III.IV
III	III	I.III
III	IV	II.III.IV
IV	IV	II.III.IV

Table shewing group of unknown parent, one parent and children known. (after Snyder)

Known children (in groups.)	One parent group.	Other parent (must be in groups
I	I	I, II or III
I	II	I, II or III
I	III	I, II or III
I	IV	Impossible *
II	I	II or IV
II	III	II or IV
III	I	III or IV
III	II	II or IV
IV	I	Impossible**
IV	II	III or IV
IV	III	II or IV
IV	IV	II, III or IV
I and II	I	II
I and II	II	I, II or III
I and II	III	II
I and II	IV	Impossible *
I and III	I	III
I and III	II	III
I and III	III	I, II or III
I and III	IV	Impossible *
I and IV	I	Impossible**
I and IV	II	III
I and IV	III	II
I and IV	IV	Impossible *
II and III	I	IV
II and III	II	III or IV
II and III	III	II or IV
II and IV	I	Impossible**
II and IV	II	III or IV
II and IV	III	II or IV
II and IV	IV	II, III or IV
III and IV	I	Impossible**
III and IV	II	III or IV
III and IV	III	II or IV
III and IV	IV	II, III or IV
I, II and III	I	Impossible**
I, II and III	II	III
I, II and III	III	II
I, II and III	IV	Impossible*

Table continued

Known children in groups	One parent group	Other parent must be in group
I, II and IV	I	Impossible**
I, II,and IV	II	III
I, II and IV	III	II
I, II and IV	IV	Impossible *
I, III and IV	I	Impossible **
I, III and IV	II	III
I, III and IV	III	II
I, III and IV	IV	Impossible *
II, III and IV	I	Impossible **
II, III and IV	II	III or IV
II, III and IV	III	II or IV
II, III and Iv	IV	II, III or IV
I,II,III and IV	I	Impossible **
I,II,III and IV	II	III
I,II,III and IV	III	II
I,II,III and IV	IV	Impossible *

In the combinations not given in this table,the other parent could be any of the four groups. On the hypothesis of three multiple allelomorphs where marked impossible with star ,neither parent could be of group IV. where marked impossible with two stars neither parent could be of group I.

From these tables,if the child or children,and one parent are known,it can be seen that a person could or could not be the other parent. The former conclusion does not carry us very far;the latter is definite and conclusive. The test may be accepted as reliable.It has been accepted as proof in courts of law on the continent.

TECHNIQUE OF BLOOD GROUPING

A. Material for tests. Blood corpuscles or blood serum of groups II and III, or in difficult cases both serum and corpuscles of these groups are required.

Test serum of known groups may be obtained commercially from Messrs Burroughs and Wellcome. Sera is also supplied commercially in Vienna, and in America the commercial product is in the dry form. Serum may however, be collected and preserved from a person whose group is known; but better still is the use of fresh serum if the observer or laboratory attendant is of the requisite group.

Serum may be preserved for days at room temperature without treatment, but heat, light and bacteria slowly destroy the agglutinin. The serum may be kept for months if sterile and kept in a cool dark place. Chemical preservatives (one part of 5 per cent phenol to 9 of serum) may be used.

Blood corpuscles should be used fresh or at the most one or two days old. Schiff calls a note of warning against the use of corpuscles which are not fresh . The test serum and corpuscles used should be sensitive. The sensitiveness may be tested by comparing a number of corpuscles of the same group against similar corpuscles and noting the rapidity of the agglutinations.

B. The blood to be tested.

In the case of living persons, that is in questions of paternity or in the examination of the blood of an accused person, fresh blood is available and the material presents no difficulty.

Fresh serum may be used, but this may cause a pseudoagglutination from the presence of haemolysins These haemolysins disappear after the serum has stood for a few days, or the disturbance may be got rid of by inactivating the serum.

Blood corpuscles must never be used concentrated owing to the risk of pseudoagglutinations. With a dilution of 2 or 3 times the original volume, the capacity for pseudoagglutinatin is completely lost.

From what has already been said, it is seen that the isoagglutination reaction always consists in treating serum of one person with the corpuscles of another. This may be done either in short test tubes or on a slide.

Test tube method. This is the better method.

A couple of drops of corpuscle free serum are placed in the tube, to this is added four drops of blood corpuscle suspension to be examined. The tube is then well shaken and centrifuged; the corpuscles are now a precipitate below a clear fluid. By carefully shaking the tube it is seen whether clumps are

formed, or whether the corpuscles are still unagglutinated. In a strong agglutination the corpuscles may form a single mass.

Glass slide method.

A drop of serum is placed on the slide and a drop of corpuscle suspension placed in contact with it. After a few minutes it is examined naked eye for evidence of agglutinitin.

Sources of Error and Controls.

Errors can be excluded with certainty if the group is determined in different ways with unknown serum and known corpuscles and vice versa; as well as control tests with blood of various origins.

False positive readings. Pseudoagglutination.

Rouleaux formation presents the same picture as isoagglutination from which it cannot be distinguished by the appearance of the clumping. It rarely occurs in the test tube method which on this account is the best for use.

With the slide method it is especially apt to occur if the corpuscle concentration is too strong or if fresh serum is used.

Pseudoagglutination may be guarded against by using diluted concentrations of corpuscles. Lattes has shewn that a true fixation of washed blood corpuscles in a 5 per cent formalin salt solution (in

which the shape of the corpuscles is not altered) or in a 5 per cent sublimate with subsequent thorough washing out, leads to a complete or nearly complete, incapacity for roleaux formation, whilst the agglutinability remains essentially unchanged. Still better, results are obtained by moderate warming of the corpuscles. Warmed in physiological salt solution, or better to avoid any excessive damaging in their own serum, for 5 minutes at 48 to 50°C, they no longer give any pseudoagglutination, whilst the specific agglutinability remains. Such warmed blood can be used for forensic purposes, in which one is using it in direct contact with a sample of the stain. A simple and perhaps still more secure method is the use of a lecithin suspension of corpuscles. Solution for this suspension is prepared as follows; 1 part of 5 per cent lecithin solution in pure ether is added to 2 parts of normal saline, warm until the ether is evaporated, shake with cold water, fill up with distilled water to the original volume, and filter.

Goroncy (104) has shewn that if a temperature of over 20°C is used and a lecithin suspension employed, all chances of panagglutin or pseudoagglutination are avoided. An additional guard in the use of serum and corpuscle controls which at once shew the presence of these conditions.

Mino described a reaction which he called pan-

agglutinatins. This was noticed especially after antibody had been fixed, and nearly always took place in the cold. It consisted in an agglutination of corpuscles of all groups including corpuscles of the same groups.

Schiff describes a further fallacy from the use of corpuscles which have been kept too long, the agglutinatin is uninfluenced by lecithin but vanishes at 37°C. Its presence would also be shewn by the corpuscle control.

False negative readings, may occur through the use of poorly acting (poorly sensitive) corpuscles or weak serum, if the slide method is used agglutinatins may take a considerable time, if however, the test tube is used and centrifuged a positive reading is unlikely to be missed.

Controls. False agglutinatins will be readily recognized if the corpuscles are tested with their own serum or serum of group IV (ABo) and if serum is tested with its own corpuscles or with corpuscles of group I (O$\alpha\beta$).

Altered blood.

The descriptions given above apply to the examinations of fresh blood from living individuals. Blood from a dead body may be treated in the same way if within a few days of death, but not after haemolysis has taken place.

Blood stains.

The success of the examination depends on the treatment to which the stain has been subjected and in some cases it may not be possible to determine the group with certainty. It is important to remember that only positive results are of value in damaged blood as properties originally present may have disappeared.

The only one of the tests already described which is of use in this case is the one depending on the serum agglutinins in the stain and known corpuscles, since the corpuscles in the stain are destroyed.

Strassman states that the stain should be tested at the earliest opportunity, as even after six weeks or less the agglutinins may have been destroyed.

Lattes cover glass method.

A very small piece of the stain is placed on a glass slide and close to it is placed a drop of corpuscle suspension, a cover glass is now placed over them and the corpuscle suspension flows round the clot and a coloured halo appears together with characteristic clumps of corpuscles. These clumps may be a positive agglutination or a pseudoagglutination due to too high a concentration of serum. If the latter, they will break up on dilution. This dilution is affected by raising the cover glass and allowing it to fall back again thus further diluting the fluid round the clot with the rest of the corpuscle suspension. If a lecithin suspension of corpuscles is

used and care taken that the temperature is not too low,(Goroncy) there is no risk of pseudoagglutinatin, and the result can be recorded at once.

Instead of the glass slide method, an attempt may be made to extract the stain and test the serum extracted by the test tube method.

Method of obtaining blood extract.

More material is needed than for cover glass method. Some of the blood clot is weighed out and extracted with four times the quantity of distilled water (Schiff) in the cold. After some hours it is centrifuged and the fluid tested for agglutinin either with lecithin corpuscles or a glass slide or in test tubes.

Examination of Blood Stains for the Corpuscular properties. (agglutinogen)

As already noted in the case of blood stains, the corpuscles are damaged and cannot be obtained as a free suspension. Testing by direct matching against known serum is therefore not available. The agglutinogens present in the stain (in A or B or both)may be determined by "fixation"tests.

Agglutinin Fixation Test.

If serum of group II,(containing agglutinin β) is left in contact with the powdered blood clot of group III (containing agglutinogen B in the corpuscles) the two unite. If the serum now removed and tested against known group III corpuscles is found

to have lost the power of agglutinating these corpuscles (i.e. it now contains no agglutinin) or that the power has been greatly diminished, the serum has lost its agglutinin which has become fixed to the agglutinogen of the clot. By warming to 54°C, the union between the agglutinins and agglutinogens can be broken and the agglutinins recovered. Their presence is then demonstrated by testing with known corpuscles.

The warning already given in regard to damaged blood must be repeated, it is only positive results which are of value. A group may not be inferred from failure to demonstrate agglutinogen by this method.

Technique of test.

The material to be examined must be reduced to a state of fine division. A control test with known corpuscles should be performed. The test sera used must contain agglutinins α and β. It is better to take equal parts of group II and III sera of approximately similar strength, rather than to use serum of group I which contains both agglutinins but in which the proportions are very variable

Schiff describes a model test, using known blood, variations of which may be made to meet individual cases.

Reagents required.

Test sera. Serum group II(β) of which 0.4cc are added to 1.6cc of normal saline making 2cc of a 1 to 5 dilution.

Serum group III(α) diluted as above.

These two sera are mixed giving 4cc of a 1 in 10 dilution of agglutinins α and β.

Corpuscles or Blood Clot. For this model test 5cc of a five per cent suspension of corpuscles are centrifuged and the deposit used.

Performance of the test.

The test sera mixture is divided into equal parts, one part is added to the corpuscles and allowed to stand in the ice chest for twentyfour hours. The tube is then centrifuged and the supernanant fluid removed (less any agglutinin fixed by the corpuscles).

The fluid treated with the corpuscles, and the untreated serum mixture are now both tested for agglutinin content, by means of corpuscles containing agglutinogen A and B.

Four tubes are taken and into tubes 1 and 2 are placed 0.2cc of the decanted(treated)serum,and into 3 and 4,0.2cc of the untreated serum mixture. Now to tubes 1 and 3 a drop of five per cent suspension of

group II (A) corpuscles is added,and to tubes 2 and 4, a suspension of corpuscles of group III(B). The presence or absence of agglutination is noted.

Tube		Agglutination.
1	0.2cc treated serum and corpuscles group II (A)	absent.
2	0.2cc treated serum and corpuscles group III(B)	present.
3	0.2cc untreated serum and corpuscles group II (A)	present
4	0.2cc untreated serum and corpuscles group III(B)	present.

Tube 1 shews that the unknown corpuscles have removed agglutinin ∝ from the treated serum.
tube 2 shews that the reaction is specific,as the normal agglutinin is not interfered with. An unspecific agent would have affected both tubes 1 and 2. In the case of group IV (AB) which contains agglutinogen A and B,both tubes will shew no agglutination.
Tubes 3 and 4 serve to control the serum and corpuscles. A slight degree of weakening of the agglutination in tubes 1 and 2 must not be taken as evidence of fixation. Only powerful or complete fixation should be recorded as a positive result.

Recovery of agglutinins.

Continuing the experiment the agglutinin laden deposit may be used. Schiff recommends the use of fresh material if sufficient is available, and the use of

a larger amount of a concentrated serum mixture, thus ensuring that the corpuscle agglutinogen takes up its maximum amount of agglutinin, and giving a greater amount for detection after release.

After standing for twentyfour hours in the ice chest, the supernatant fluid is removed, and the corpuscles well washed in normal saline. A small amount of ice cold saline is then mixed with the corpuscles which are again centrifuged, the saline being removed and tested for agglutinin, if any is found the washing has not been sufficient and must be repeated till all traces of agglutinin have been removed. When the washings are free from agglutinin, a small amount of saline is added to the corpuscles, and the tube heated for five minutes at 54° C. and then quickly centrifuged. Schiff centrifuges the tube in a larger tube filled with water at 54° to avoid cooling. The supernatant fluid is then removed, and tested against corpuscles of group II (A) and group III (B) for the presence of agglutinins α and β.

Sources of error in the fixation and liberation test.

1. A failure of fixation owing to the agglutinogen being too weakened by age or other influences. This is guarded against if only positive results are accepted.
2. Confusion of specific with unspecific fixation. In unspecific fixation both agglutinins are affected, whereas in specific only will be influenced, the

other acting as a control. In the case of group IV(AB) this check is not present as both agglutinins are fixed.

SUMMARY OF FORENSIC APPLICATIONS OF BLOOD GROUPING.

The uses of blood grouping have already been indicated in general, it now remains to consider the practical deductions which can be drawn from such examinations.

1. Cases of paternity.

The blood groups of the child and mother being ascertained, the group or groups to which the father may belong can be predicted with certainty (see page 100). If the suspected father does not belong to one of these groups, then suspicion is removed from him, he cannot be the father. If on the other hand he belongs to one of those groups, it indicates that it was possible for him to be the but not that he was the father. This test can be relied upon, and has already been accepted in courts of law on the continent.

2. Legitimacy, and substitution of children.

The same remarks apply as in the case of paternity, except that the blood groups of children in the first year of life can only be determined by the reactions of the corpuscles, the agglutinins in the serum being insufficiently developed at birth.

3. Assaults.

The statement that the blood on a person's clothes is from themself (from nose,cut hand,etc.) may be disproved.

As in this case we are not examining fresh blood the test may fail from weakening of the agglutinin due to aging or other reasons which are described under the examination of stains.

MENSTRUAL BLOOD.

A common defense in cases of assaults on females is that the blood on the woman's garments is due to menstruation. Menstrual blood gives all the ordinary reactions for blood.

Sydney Smith (105) states that on microccopic examination after staining, menstrual blood may be daignosed by the presence of endometrial cells of the uterus and by vaginal epithelial cells, in addition to large numbers of bacilli and cocci.

I do not agree with this statement. I admit that these characters may be found in menstrual blood and that ordinary blood clot is free from large numbers of microorganisms and cells, but looking to the state of the garments which one is accustomed to receive for examination in these cases, there is no reason to suppose that they were not already saturated with vaginal discharges. In any case, an act of rape or even the irritation from an indecent assault frequently causes an innocent watery discharge which, together with the blood from another source, may cause the presence of these cells and organisms in the blood stains.

I consider that no evidence can be given as to

whether blood is of menstrual origin or not. The most that can be done, is to infer from the position and extent of the stain, that the blood is probably the result of menstruation.

DETERMINATION OF SEX OF BLOOD STAINS

The determination of sex stands in close connection with the hormones of the sex glands. In each sex peculiarities of the blood depends on the hormones. The materials circulating can be detected and chemically differentiated.

Dewitz first used a chemical reaction for determining the sex in butterfly pupae. Manoiloff (82) claims to have now shewn the possibility of sex distinction by chemical reaction. He gives three modifications into which from 70 to 96 per cent fall.

I. Modification gives 70 positive per cent, and is carried out with the following reagents.

i. 0.5 per cent peroxide solution.

ii. 1.0 per cent watery solution ninhydrin 8.+ 10.0 per cent, potash 2.0.

iii. 1.0 per cent watery solution methyl violet (1912).

All these reagents are prepared at the time, and then the reaction carried out in the following way.

To 2.0 cc unheated blood serum is added of reagent i. three drops. reagent ii. five to six drops and after a little shaking, of reagent iii, three drops The mixture is now shaken slightly and placed on one side for from a quarter to half an hour. With male blood a considerable colour quickly appears, whereas,

with female blood there is no colour or only a very slight tinge.

In the second modification the following reagents:-

i. 1.0 per cent permanganate solution,

ii. 0.5 per cent hydrogen dioxide,

iii. 40.0 per cent aqueous chloride of potash,

iv. 1.0 to 2.0 per cent aqueous thiosinamine,

v. 10.0 per cent aqueous potash,

vi. methyl violet (1912) or dahlia 2.0 per cent.

The method used for the second modification is to add to the blood serum, i. 5 drops, ii. 3 drops, iii. 3 drops, iv. 5 drops, vi. a single drop if necessary, shaking on each addition. Male blood is heavily coloured compared with female and positive results in 70-80 per cent.

A third modification,he states gives 86-96%results. The third method, (tested on 530 cases). He uses,

i. 1.0 per cent watery solution of papayotinum (Merck).

ii. alcoholic dahlia in 1.0 per cent solution.

iii. 1.0 per cent aqueous permanganate.

iv. 40.0 per cent aqueous KCl.

v. 2.0 per cent thiosinamin.

To 3 cc of a 10.0 to 30.0 per cent erythrocyte emulsion there is added,i. 10 drops and after 1 to 2 minutes add reagent ii. 3 drops then reagent iii. 10 drops. After this shake carefully and then add reagent

iv. 3 drops and reagent v. 5 drops, after each addition shaking carefully.

He found that in a pregnant cow he got the reaction of male blood but that was the foetal sex, and suggests that the same may possibly happen with human blood. He here, adds after solution ii. some aqueous 1.0 per cent solution aesculin (Merck) 5 to 6 drops.

In 436 cases, blood from the navel gave 88 per cent positive results. He also states that the test may be used forensically.

In the case of human blood on the clothes of a man accused of murdering a woman, or vice versa, a positive male reaction would obviously be of great forensic value, and even were only 90 per cent of the results correct it might still be an aid. Further investigation however, is required not only into the accuracy of the test, but also into the effects which the age of the stains may have on the results before it can be of any practical forensic value.

DIFFERENTIATION OF RACE

Attempts to differentiate the race to which an individual belongs by an examination of the blood have been made by Manoâloff (81), he adds 3 cc of a 3-5 per cent emulsion of blood cells (obtained from the clot) one drop of a 1 per cent alcoholic solution of methylene blue. After stirring, he adds 5 drops of a 1 per cent alcoholic solution of cresyl violet and stirs again. He adds 3 drops of a 0.5 - 1 per cent solution of silver nitrate and, after stirring, 1 drop of a 40 per cent solution of hydrochloric acid. After stirring well, he adds 3-5 or more drops of a freshly prepared aqueous 1 per cent solution of potassium permanganate. The amount of the latter depends on the quality of the dyes. He finds that the cresyl violet disappears in the blood taken from Jews, which becomes blue, while the fluid stays bluish red with blood from Russians. He had 187 correct results in 202 tests. He found differences even between the blood of Russians and Ukrainians. The children from mixed marriages (father Russian, mother Jewish, Polish or Armenian) gave a faster reaction than pure Russians. No difference from the Russian was noted in children from intermarriage between Russians and the German or yellow race.

Kogan observed with this method that injection of epinephrine induces temporarily the "Russian" reaction in Jewish children. He explains the differences by

greater speed of oxidation in Jewish blood as compared with the Russian.

Whilst of scientific interest, this has at present no practical forensic value.

DETERMINATION OF AGE BY EXAMINATION OF THE BLOOD

Hajek (94) attempts to estimate the age of an individual by examining the blood, but Goroncy (95) found his method too unreliable for forensic work.

I have no experience of this method. Goroncy states that Hajek based his work on Ruzicka's on protoplasmic hysteresis, by which is understood a progressive change of the living substance with age, similar to other ageing of colloids, and consisting in an important diminution of solubility. According to Ruzicka, the degree of this alteration furnishes a measure of the age of the tissue.

Of the numerous methods put forward by Ruzicka, Hajek has used two for his work on blood- precipitation by alcohol, and measurement of the hydrogen ion concentration. Tested by Marx, this was found unreliable for forensic purposes.

Hajek examined blood of the cadaver taken from the heart and from the dural sinus. He allowed the blood to dry in air, and then dissolved 0.1 in 200.0 distilled water, and determined the H Ion concentration.

Out of 120 cases, he found the concentration to be pH 7.65 to 6.1. Combining each five years of life in a curve, the first year shewed about pH 7.0,

to 7.5, and the forty-fifth year pH 6.54. The curve then began to rise slightly again. The diminution is regular through life, and Hajek believes that it falls in correspondence with age. He calculated a standard value for dried blood, (pH 6.77 for ages between 35–45), and as a tentative suggestion gives pH above 6.7 for individuals below 35. If pH is less than 6.8, then the individual is over 45.

Before considering dried blood, it should be stated that earlier observers found variations of pH from 7.1 to 7.45 at all ages independent of age.

In determining the pH value, Goroncy found the CO_2 tension of the highest importance. As in pathologic conditions i.e., diabetes, with its acetic and β-oxy-butyric acids, the relations of alkali and carbonic acid are changed. He dried blood in a thin film in air. After two or three days, when quite dry, the blood was diluted to forty times its original volume and its pH value tested. He found that the pH did not follow any law with respect to age, and did not correspond at the same ages. He concluded that the whole process was too unreliable for any forensic purpose.

HAEMOGLOBIN AND ITS DERIVATIVES.

In addition to the examination of a stain to obtain proof of blood, it may be necessary to examine known blood, obtained from a body, to determine the compound of haemoglobin present, as for instance in cases of carbon monoxide poisoning, poisoning from shoe black, petrol vapour, etc., and in the investigation of such cases it is frequently necessary to prepare control solutions in the laboratory.

Most textbooks on Physiology consider the derivatives of haemoglobin, but of necessity only essentials are set out , some of the statements are inaccurate, and the subject is treated from the academic rather than the medicolegal point of view.

When the medicolegal question of the identity of a haemoglobin derivative is under consideration, much detail may be required, its presence confirmed by several methods, and in some cases its percentage in the bloodhas to be estimated.

I have found on reference to a number of monographs that on these points many contradict one another.

In an attempt to obviate this it is proposed to consider here, the diagnosis, and the circumstances under which each particular derivative of haemoglobin may occur, as well as their method of preparation in the laboratory, at the same time indicating the literature

Except in a few cases mentioned I have personally examined each spectrum, and performed each test, and mode of preparation or estimation. The bands of the

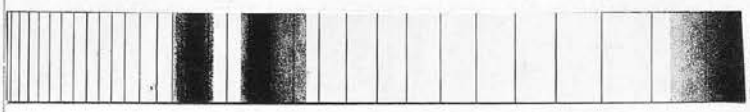
various spectra have all been carefully measured by the microspectroscope, and found to correspond to those given by Dilling (10) which being taken with a more accurate instrument are the values quoted.

OXYHAEMOGLOBIN

Oxyhaemoglobin is of a bright red colour, and is the compound present in freshly shed blood in contact with air.

It is insoluble in ether or chloroform, but freely soluble in water, and is precipitated from the watery solution by many metallic salts.

The spectrum shews two absorption bands between D and E. The band nearest the red being the darker, narrower, and sharper and almost touching the D line.



Wavelengths
589-567(578-5)
553.5-525(542.0)

Oxyhaemoglobin

On addition of a reducing agent such as sodium hydrosulphite, yellow sulphide of ammonia, or Stokes* solution, these two bands are replaced by the single band of reduced haemoglobin, which on vigorous

*Stokes Reagent. Asolution containing 2 per cent of ferrous sulphate, and three per cent tartaric acid. When needed for use a small amount should be placed in a test tube and ammonia hydroxide added until the precipitate which forms on the first addition of the hydroxide has entirely dissolved. This produces ammonium ferrotartarate which is a reducing agent.

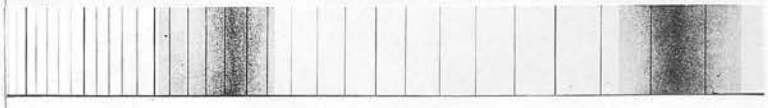
shaking with air again shews the reoxidation to oxyhaemoglobin. On standing the oxyhaemoglobin becomes reduced again. This interchangeability with the resulting change of spectrum and colour, is diagnostic.

HAEMOGLOBIN. (REDUCED HAEMOGLOBIN).

Haemoglobin is easily soluble in water, the solution being of a purplish colour.

It is obtained when a reducing agentsuch as Stokes solution,ammonium sulphide, sodium hydrosulphite, or hydrazine sulphate is added to oxy- or methaemoglobin.

The spectrum shews a single ill defined band of absorption between D and E. The band may extend a little to the red side of D . The other margin reaching as far as E .



Wavelengths
596.5-536(555)

Reduced haemoglobin

If prepared by the use of ammonium sulphide, or sodium hydrosulphite, a narrow band may be seen in the red at 62 due to the formation of a sulphur compound of haemoglobin.

It is importantthat the ammonium sulphide should be old, and contain no free ammonia, or the spectrum of haemochromogen will be obtained.

On the addition of an oxidizing agent or of water, or on shaking with air,haemoglobin is converted into oxyhaemoglobin. On treating with dilute alkali, the

characteristic spectrum of haemochromogen is obtained, the colour changing to cherry-red.

Gamgee (63) states that nitrites and potassium ferricyanide have no action on reduced haemoglobin, on the other hand Haldane (66) states that methaemoglobin is formed. This may be so in the case of haemoglobin obtained from oxyhaemoglobin by means of a vacuum pump. The statement is apt to mislead anyoneattempting to prepare solutions for comparison, as if the haemoglobin is formed by the reduction of oxyhaemoglobin, that is if a reducing agent is present in the solution nitrites immediately form nitroxyhaemoglobin (q.v.) and potassium ferricyanide forms haemochromogen.

METHAEMOGLOBIN. (NEUTRAL METHAEMOGLOBIN).

This is a soluble substance of a dirty reddish brown, which gives the almost black colour of decomposing blood.

It consists of oxygen and haemoglobin combined in a different manner to that found in oxyhaemoglobin; it occurs when blood is exposed to air and light. It may even occur in the blood and urine during life in certain cases of poisoning by antipyretics, potassium chlorate, amyl nitrite, oxalic acid, etc. Cases have occurred from aniline used in patent shoe polishes, Lloyd (4), Dettrich (5), and six cases are recorded by Gordon Watson(6. 7. 8.) which resulted from the bursting of a high explosive shell in a confined space. It may also occur in haemorrhagic transudates, and is the

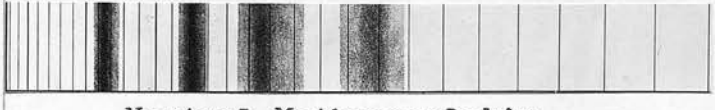
condition found in one of the forms of enterogenous cyanosis.

METHAEMOGLOBIN.

Is readily prepared by acting on oxyhaemoglobin with such agents as potassium ferricyanide, potassium permanganate, nitrites or very dilute acids.

It exists in the two neutral forms, neutral and alkaline, which are interchangeable by varying the reaction of the medium. The neutral form is the one obtained by the above methods, and I find that the best alkali for converting this into the alkaline form is weak ammonia.

SPECTRUM of neutral methaemoglobin shews a band in the red, and two bands in positions similar to those of oxyhaemoglobin. A fourth faint band may be seen in the green. This spectrum is probably a mixture of oxy and methaemoglobin.



643.5-623 (634)
587-571 (578.5)
555-526 (542)
512-492 (500)

Neutral Methaemoglobin

ALKALINE METHAEMOGLOBIN

Is obtained when a few drops of weak alkali (ammonia) are added to neutral methaemoglobin. The solution becomes red in colour, and the absorption spectrum changes shewing a band on either side of the D line, the band nearer the red being fainter. A third band is seen corresponding to the B band of oxyhaemoglobin, and a fourth band may be seen in the blue green.

To see the bands well the solution should be viewed in considerable depth as the bands are liable to be weak.

616.5-593(602)
586.5-567.5(578)
550-528 (541.5)
500-478 (492)

Alkaline Methaemoglobin

On the addition of a reducing agent methaemoglobin is reduced to haemoglobin except when prepared by addition of a nitrite, when it retains its red colour and is converted into nitrooxyhaemoglobin.

HAEMATIN (oxyhaematin)

Haematin is amorphous, only slightly soluble in water, insoluble in excess of dilute acids, and so is precipitated by excess of acid, insoluble in alcohol, ether and chloroform. It dissolves in warm glacial acetic acid, acid alcohol, acid ether, and in alkalis even when very dilute, but is resistant to boiling sodium hydroxide and boiling hydrochloric acid. It exists in two forms, acid and alkaline, which are interchangeable. The acid solutions are always brown in colour whilst the alkaline solutions in transmitted light, appear green in thin layers, and red in thicker layers.

Haematin is obtained by the action of weak acids or alkalis on oxyhaemoglobin, haemoglobin, or methaemoglobin, the haemoglobin being split up into globin and haematin, (see page),thus the blood in

cases of haemorrhage into the stomach or intestines is converted into haematin by the gastric or intestinal juices. In the same way the black brown appearance of the stomach in the case of certain poisons, is due to the formation of haematin. It is said to be found in the urine after poisoning by arseniuretted hydrogen (Hammerstein 21).

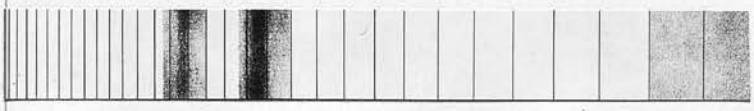
On treatment with strong sulphuric acid, the iron is removed, and haematoporphyrin is formed. On the addition of a reducing agent to an alkaline solution, it is reduced to haemochromogen which has a cherry red colour.

Haematin does not give the reaction for iron, and in this way is easily differentiated from rust stains. THE SPECTRUM of haematin varies with the strength, reaction and medium in which the haematin is dissolved. In all cases it is far from delicate, and on this account is not used as a proof of the presence of blood, though Petterson and Haines (50) refer to the spectrum as characteristic.

The following spectra may be obtained

ALKALINE HAEMATIN in Aqueous Solution

This shews two ill defined bands between D and E, with absorption of light at the violet end as far as the green.



593-570(581.5)
552.5-530(544.5)

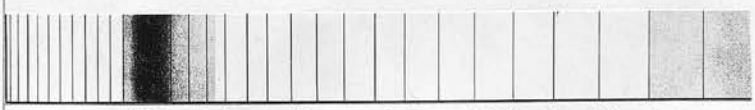
Alkaline Haematine (aqueous)

It is obtained when caustic soda is added to a one per cent solution of blood. If the above solution is heated and then diluted with water, the two bands are gradually replaced by a single band 630-565 with two maxima of absorption at 620 and 581.

ALKALINE HAEMATIN in Alcoholic Solution.

This is obtained by adding to the aqueous solution an equal volume of alcohol.

The spectrum shews a single band to the red side of D , with absorption of the violet and most of the green



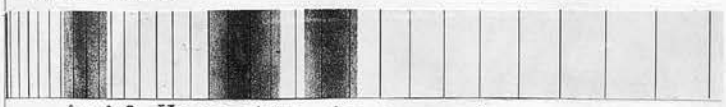
620-565 (600)

Alkaline Haematin (Alcoholic)

ACID HAEMATIN in Aqueous Solution.

On adding a drop of dilute acid to diluted blood, a brown red solution of haematin is produced. On further addition of acid the haematin is thrown down as an amorphous precipitate.

In strong fresh solutions two ill defined bands will be seen near the E line, and one faint band in the red.



668-633(653.5)
570-536 (556)
526-507.5(516.5)

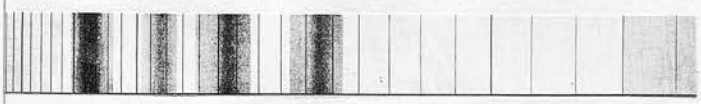
Acid Haematin (aqueous)

Sutherland states that in older solutions these bands will be found at 66.5, 56.5, and 52.6.

ACID HAEMATIN in Alcoholic Solution.

This is produced when one part of diluted acid and two parts of ether are added to oxyhaemoglobin.

It shews a well marked band in the red, and a broad poorly defined band between D and F, which on dilution resolves into one faint band to the red side of E, and another in the green. A fourth very faint band may sometimes be made out to the violet side of D.



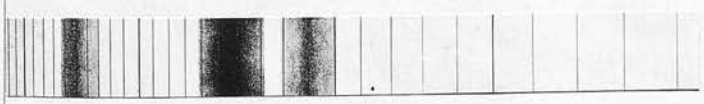
652-617 (638.5)
596.5-573.5(583)
561.5-533.5(544.5)
516.5-496.5(505.5)

Acid Haematin (Alcoholic)

HAEMIN.

The spectrum obtained when pure haemin is dissolved in glacial acetic acid is described as that of haemin, by Dilling (10).

It shews a band in the red, two in the green, and one in the violet.



662-630 (649.5)
561-528.5(544.5)
521-500 (511)
395-367 ( )

I have not been able to obtain this spectrum from haemin crystals with the microspectroscope, but have obtained the bands on adding potassium ferricyanide to old blood.

HAEMOCHROMOGEN (Reduced Alkaline Haematin)

This is a cherry red substance containing all the iron of the haemoglobin. It is obtained by the addition of a reducing agent to alkaline haematin, or by addition of an alkali and reducing agent to oxyhaemoglobin.

It has a characteristic spectrum, which constitutes a most reliable test for blood.

The spectrum shews two absorption bands, one very sharp, dark and well defined, between D and E, the other broader and fainter at E.

Haemochromogen

Crystals of haemochromogen are readily obtained and form a conclusive proof of the presence of blood. (See haemochromogen crystal test).

So called acid haemochromogen is described but is hard to obtain. It results when haemoglobin is decomposed by acids in the presence of strong reducing agents and shews four bands, which according to Jaderholm (61) depend on the mixture of haemochromogen and haematoporphyrin due to further decomposition of part of the haemoglobin.

HAEMATOPORPHYRIN (Iron free Haematin).

On the addition of strong sulphuric acid to blood haematin or other derivatives of haemoglobin, the iron is split off and haematoporphyrin is formed.

It exists in acid and alkaline forms, which have different spectroscopical appearances, and which are interconvertable by altering the reaction of the medium.

This is of use in proving the presence of blood in cases of stains where the blood has been converted into haematin or haematoporphyrin.

Traces occur normally in the urine, and large amounts are found in cases of sulphonal poisoning. In these cases it is said to be usually in alkaline form.

It is insoluble in water, soluble in acid, alkali or alcohol, and is said to be isomeric with bilirubin. Like this latter it gives the play of colours with fuming nitric acid.

Halliburton (12) states that it may be reconverted into haematin by heating with Stokes's solution after rendering the solution alkaline with ammonia and adding a few drops of a reducing agent such as hydrazine hydrate.

Milroy (3) heats with ferrous acetate. He also outlines a simple scheme for demonstrating the con-

version of haematin to haematoporphyrin and back to haematin again. This operation is not easily accomplished. The presence of any free acids or excessive heating prevents the union of the haematoporphyrin with the iron.

ACID HAEMATOPORPHYRIN.

Its solutions are of a purple colour having a red fluorescence, and it may be obtained in the solid form as a dark purple powder by the addition of excess of water to the acid solution. The precipitate dissolves in alkali to form the alkaline haematiporphyrin.

The spectrum has a narrow faint band in the red, and a second darker band between D and E.

Acid Haematoporphyrin

On shaking up the acid solution with chloroform a spectrum shewing five bands, of which two are in the red is often obtained. These bands are well seen and are very persistent.

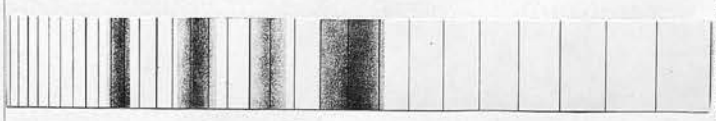
ALKALINE HAEMATOPORPHYRIN.

This is obtained on the addition of strong alkali to the acid solution. It is best prepared by almost neutralizing the acid solution with sodium hydroxide, when a precipitate separates out. The precipitation may be rendered more complete by the addition of sodium acetate. Filter off and dissolve in dilute sodium hydroxide. If on a microscope slide, wash the clot of

acid haematoporphyrin by running in water under the cover glass, then run in alkali and the clot will be gradually converted to the alkaline form.

The solution is of a red colour, shewing four bands in the spectrum. The faintest band and first to disappear on dilution is in the red midway between C and D . A second, broader and darker band lies on the violet side of D ; a third between D and E; and a fourth, broad and dark at the junction of the green and blue.

On the addition of strong acid this is reconverted to the acid form.



Alkaline Haematoporphyrin

Skand (14) and Garrod (15) state that on the addition of a solution of zinc chloride in excess of alkali the spectrum changes rapidly to one with two bands, one of which surrounds D, and the other lies between D and E. I have not been able to obtain this spectrum

CYANMETHAEMOGLOBIN (Photomethaemoglobin)

When sunlight is allowed to act on methaemoglobin solution containing potassium ferricyanide or traces of cyanide the colour changes to red and in neutral or acid solutions a spectrum similar to that of haemoglobin is obtained. Dilling states there are two maxima of absorption and Balthazard one. I have

however not been able to determine any marked maximum.

This substance was called photomethaemoglobin by Bock who first described it. It is frequently called cyanhaemoglobin but Balthazard and Martin (70) state that this is a different substance.

It is formed at once when potassium cyanide is added to methaemoglobin in the cold, but is not formed directly from oxyhaemoglobin unless kept at body heat. This is the compound formed in poisoning by cyanides.

Cyanmethaemoglobin

Reducing agents, other than ammonium sulphide which contains alkali, convert it to reduced haemoglobin, but with an excess of potassium cyanide an intermediate substance is found having two sharp dark bands at 565 and 537. . They are similar to those of cyanhaemochromogen but very slightly nearer the red. This is the substance which Balthazard calls cyanhaemoglobin; it is unstable and quickly turns to reduced haemoglobin.

In the presence of an alkali reducing agents carry the process further converting the cyanmethaemoglobin into cyanhaemochromogen.

CYANHAEMOGLOBIN see Cyanmethaemoglobin.

CYANHAEMATIN

This is obtained by adding potassium cyanide to

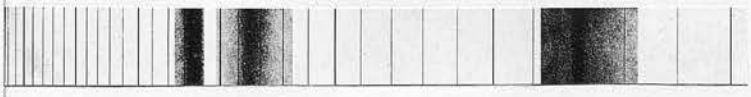
alkaline haematin or by the addition of sodium hydroxide to cyanmethaemoglobin. The colour and spectrum are similar to those of cyanmethaemoglobin. They can readily be distinguished by their behaviour with a reducing agent; cyanmethaemoglobin being finally reduced to haemoglobin, especially on warming; cyanhaematin on the other hand is at once changed to a pinkish red in colour, and the striking spectrum of cyanhaemochromogen is seen.

595-525 (552.5)

Cyanhaematin

CYANHAEMOCHROMOGEN.

This is of a cherry pink colour; it shews two well marked bands in the green, very similar to those of haemochromogen but broader and slightly nearer the red



570
534

Cyanhaemochromogen

It forms a valuable test for blood, especially in hot climates. Lyon and Waddell in India used it as a routine method (17).

It may be prepared by adding potassium cyanide to haemochromogen, or a reducing agent to cyanhaematin, or an alkali and reducing agent, ammonium sulphide is both, to cyanmethaemoglobin.

CARBON MONOXIDE HAEMOGLOBIN. (CARBOXYHAEMOGLOBIN) see also appendix"A"

This is a much more stable compound than oxyhaemoglobin and is bright pink in colour. It is met with in cases of gassing in mines, in lighting gas poisoning, gas from burst mains filtering through floors of houses, explosions, brick kilh fires and in poisoning from the exhaust gases of motor cars etc. In connection with the latter, Yandell Henderson (22) states that from a 20 h. p., car there is enough carbon monoxide given off to render the atmosphere of a small closed garage, 10x10 by 20 feet deadly in less than ten minutes.

Carboxyhaemoglobin is easily prepared by passing coal gas through a solution of normal blood. If undiluted blood is used this takes some time, but if diluted, the blood readily takes up the carbon monoxide especially if a drop of ammonia or a little saponin is added to complete the laking of the corpuscles.

Carboxyhaemoglobin is decomposed by acids and alkalis in the same way as oxyhaemoglobin, giving CO-haematin (carmine red) which on reduction becomes carboxyhaemochromogen, (see below). It is on this reaction that most of the qualitative tests for CO-haemoglobin depend; the pink colour of carboxyhaematin contrasting strongly with the brown black colour of ordinary haematin.

Spectrum

Carboxyhaemoglobin shews two absorption bands between D and E, almost in the same position as those of oxyhaemoglobin, but closer together and slightly nearer the purple.

Spectrum of Carboxyhaemoglobin.

TESTS.

It is distinguished from oxyhaemoglobin by the fact that on addition of a reducing agent no change takes place in the spectrum, whilst the bands of oxyhaemoglobin are replaced by the single band of reduced haemoglobin. Zaugger (87) notes that owing to decomposition, blood which contains no carbon monoxide may fail to reduce, the two bands of oxyhaemoglobin being permanent. This I think is a wrong deduction and in all probability nitroxyhaemoglobin (q. v.) had been formed in the blood by nitrifying organisms.

In many cases when the percentage saturation with carbon monoxide is low, a mixed spectrum is obtained with a reducing agent, shewing the single broad band of haemoglobin with two dark bands of CO-haemoglobin superimposed on it. Douris (65) states that $\frac{1}{7}$th of the blood must be affected (14 per cent saturation) before a diagnosis can be made by the spectroscope.

CO haemoglobin may be further distinguished from oxyhaemoglobin by chemical tests. The following are those most commonly in use.

DILUTION 1. On dilution with water it retains its pink colour. Oxyhaemoglobin becomes golden yellow.
2. Hoppe Seyler's test. To a 10 per cent solution of blood add half its volume of strong caustic soda. Carbon monoxide blood gives a bright red coagulation of carboxyhaematin, normal blood gives a dark brown colour.
3. Katayama's test. To 5 cc of blood diluted 1 in 50 add one cc of yellow ammonium sulphide and 2-3 drops of 30 per cent acetic acid. CO blood gives an immediate light pink, and normal blood a green precipitate. Potassium ferrocyanide may be used in place of ammonium sulphide in the above test, giving a dull pink and a mud coloured precipitate respectively.
4. Kunkel's tannic acid test consists in adding to blood diluted with ten parts of water, a small amount of 3 per cent (or stronger) tannic acid solution. In the presence of CO-haemoglobin a pink precipitate is formed, whereas normal blood gives a dark brown coagulum. The difference is more marked on standing.

When potassium ferricyanide is added to carboxyhaemoglobin in daylight, methaemoglobin is formed. Hartridge (68) has shewn that this action is due to actinic rays present in sunlight as the reaction does

not occur in the dark and only very slightly in electric light owing to its poorness in these rays. In daylight then, the following modification of Kunkel's test as described by Hawke[13], may be carried out.

Divide the blood into two parts and dilute each with 4 times its volume of water. Add to each an equal amount of 10 per cent potassium ferricyanide, allow to stand for a few minutes, removing the stopper occasionally to allow the carbon monoxide to escape and air to enter. To each then add an equal amount of ammonium sulphide and 10 per cent tannic acid solution, the one that has been shaken and so converted from carboxy- to oxyhaemoglobin will shew a dirty brown precipitate, whilst the one containing carbon monoxide will give a pink coagulum. This test is said to be more delicate than the spectroscopic test and serves to detect as low a content as 5 per cent of carboxyhaemoglobin.

Hawkes method has the advantage that it obviates the necessity of obtaining normal blood as a control.

BOILING. Blood in strong solution boiled gives a brick red mass with CO blood and a brown coagulum with normal blood. If dilute solutions of blood are used the pink carboxyhaemochromogen is decomposed into ordinary haematin and the reaction for normal blood is obtained. (see nitroxyhaemoglobin). The above tests are given by nitroxyhaemoglobin, the differentiation from which is given under that heading

CARBOXYHAEMOCHROMOGEN. (Reduced carboxyhaematin) is of a bright red pink colour and gives the same spectrum as carboxyhaemoglobin. Unlike CO haemoglobin however, it is very unstable in the presence of oxygen, being readily converted into brown coloured (oxy) haematin. It may be obtained by adding a reducing agent and an alkali to CO blood, or by passing CO through a solution of haemochromogen.

SULPHAEMOGLOBIN is not found in the blood in cases of poisoning by sulphuretted hydrogen even when a gross excess of the gas is present, the hydrogen sulphide being readily oxidized to relatively non-toxic substances in the body. The features of acute fatal cases are simply those of pure asphyxia; but the gas also acts as an irritant to the respiratory tracts. (Haggard 26).

It has not been possible to produce sulphaemoglobin experimentally in warm blooded animals by inhalation of sulphuretted hydrogen (Hamilton 73). This suggests that the action of some intermediary substance is essential for its production. The condition occurs clinically in some forms of enterogenous cyanosis and in this connection was first described by Van der Bergh (28) who differentiated it from methaemoglobin by the addition of ammonium sulphide. (see below).

Sulphaemoglobin may be formed in vitro by the action of hydrogen sulphide on blood, but only after the

blood has become reduced and then only in the presence of concentrations of the gas far above those that would be instantly fatal if inhaled. (Haggard).

Powerful reducing agents when present greatly accentuate the production of sulphaemoglobin in blood treated with minute amounts of hydrogen sulphide, and this suggests that the hydrogen sulphide normally present in the bowel is sufficient to cause this condition when a reducing substance is also present.

Mackenzie Wallace (27) and Garrod and Gordon (31) have demonstrated a nitrate forming bacillus in the saliva in these cases which they have called the nitroso bacillus. Van der Burgh (28) has also demonstrated organisms in the stools which were capable of transforming haemoglobin into sulphaemoglobin, the blood becomes purplish in colour and develops in addition to the bands similar to oxyhaemoglobin, a third band in the red slightly nearer the yellow end of the red than that seen in methaemoglobin.

Spectrum of sulphaemoglobin.

The band in the red is seen after a few minutes and increases in intensity; it is first associated with one broad band correcponding to that of reduced haemoglobin, on standing, this broad band is slowly replaced by two bands corresponding in position to

those of oxyhaemoglobin. After some considerable time a greenish precipitate forms which can be removed by filtration.

Sulphaemoglobin remains stable for a considerable time, but even small amounts of acid convert it into acid-haematin. It is only produced, except pathologically, by substances which contain sulphur and which are also reducing agents.

Wood Clarke (29) states that a small piece of sodium hyposulphite causes an immediate reduction of oxyhaemoglobin to haemoglobin, and on standing a band appears in the red and the single band of haemoglobin is slowly changed to two bands approximating in position to those of oxyhaemoglobin, giving the spectrum of sulphaemoglobin. I have not been able to obtain this result within twenty-four hours, so this reaction is unlikely to cause any fallacy in medico-legal work.

The spectrum of sulphaemoglobin may be confused with that of methaemoglobin. It can be distinguished from the latter by the addition of ammonium sulphide.

Methaemoglobin is converted into reduced haemoglobin in the case of sulphaemoglobin the band in the red remains, but the other two bands (if excess of hydrogen sulphide is used) are replaced by one broad band.

Wood Clarke and W.H.Hurtly (29) describe a test which they say is characteristic. When carbon monoxide is passed through sulphaemoglobin all the bands are shifted towards the violet. The band in the red moving from 610-625 to 605-620. The other two bands moving to the position of those seen in carboxyhaemoglobin. The movement of the band in the red is very evident without measuring the wave length but that of the other two I found was not so readily noticed. To this spectrum they give the name carboxysulphaemoglobin.

This change does not occur in the case of methaemoglobin. Carboxysulphaemoglobin may also be obtained by passing hydrogen sulphide through a solution of carboxyhaemoglobin.

Hoppe Seyler (30) who first described sulphaemoglobin stated that it could be produced from reduced haemoglobin, but this was due to the fact that he used carbon dioxide to reduce the oxyhaemoglobin. Blood so reduced will not form sulphaemoglobin.

NITROXYHAEMOGLOBIN.

This compound is formed when nitric oxide is brought into contact with reduced haemoglobin. It was shewn by Haldane that the red colour of raw salted meat was due to nitroxyhaemoglobin, whilst the pink colour of cooked salted meat was due to nitroxyhaemochromogen, (62). This being due to the formation of a nitrite from the nitre used in salting. He also shewed that in animals killed after receiving a dose of nitrite the blood becomes bright red after death, consequent upon the formation of nitroxyhaemoglobin (18), and that this might be mistaken for carboxyhaemoglobin poisoning.

If a microorganism produces nitrite in the body the haemoglobin will be converted into nitroxyhaemoglobin after death.

Many bodies of soldiers dying from influenzal pneumonia in the 1918 epidemic were found to have a red colour although during life they had been cyanosed . Possibly methaemoglobin had been converted to nitroxyhaemoglobin. (Haldane and Banham .19.)

NITROXYHAEMOGLOBIN.

Poisoning may occur by the gases of high explosives, motor car exhausts, etc. Haldane (19)reports a case evidently caused by stoking furnaces.

When nitric oxide, or a nitrite*, is added to oxy-

*Amyl nitrite when present in excess produces photomethaemoglobin, not so the other nitrites.

oxyhaemoglobin it is first converted into a nitrite, this in turn forms methaemoglobin; on furtheraddition of nitric oxide or nitrite the methaemoglobin is reduced and converted into nitroxyhaemoglobin. Again, if a nitrite is added to oxyhaemoglobin, methaemoglobin is formed which on addition of a reducing agent is not reduced to haemoglobin but retains a red colour, and is converted into nitroxyhaemoglobin.

If on the other hand the methaemoglobin is boiled a bright pink coagulum of nitroxyhaemochromogen is formed

Haldane states that both the colour and spectrum indicate that nitroxyhaemoglobin is a compound analagous to carboxy- or oxyhaemoglobin, and does not agree with Anson and Mirsky (67) who claim that it is a compoundof nitric oxide and methaemoglobin. Dilling (10) describes a spectrum with a third band in the red, which he calls nitrimethaemoglobin, and which he obtains by adding some drops of potassiun nitrite to methaemoglobin obtaining a change of colour to red. I have obtained this spectrum but consider that it is probably a mixture of met- and nitroxyhaemoglobin. On the addition of a reducing agent the band in the red disappears the whole being converted into nitroxyhaemoglobin

Nitroxyhaemoglobin may be prepared by adding ammonia to blood and then passing a stream of nitric oxide through the solution. Addition of ammonia is necessary

to neutralize the nitric acid, formed by the action of nitric oxide and water, which as Gamgee points out would otherwise decompose the haemoglobin.

It is more easily prepared by adding solid sodium nitrite and sodium hydrosulphite to oxyhaemoglobin. nitroxyhaemoglobin being formed at once, the solution turning a deep red. It is not advisable to use ammonium sulphide as a reducing agent in this method as it forms a yellow precipitate with the sodium nitrite.

Nitroxyhaemoglobin is of a deep red colour, midway between that of oxy- and carboxyhaemoglobin. It gives a spectrum similar to that of oxyhaemoglobin but the bands are less sharply defined and the a band extends further towards the red end of the spectrum, possibly due to traces of reduced haemoglobin present in the solution.

Anson and Mirsky (67) distinguish an acid and alkaline form of nitroxyhaemoglobin which they say are interconvertable; the acid form being more distinct with its a band at 568.7

Like carboxyhaemoglobin the spectrum is unaffected by reducing agents.

On boiling, or on the addition of sodium hydroxide, a dark pink coagulum of nitroxyhaemochromogen is formed. Unlike carboxyhaemochromogen this compound is stable, and not decomposed by heat, and in this way the

nitroxyhaemoglobin can be distinguished from carboxy-haemoglobin which is decomposed, a brown precipitate of haematin being formed. Care must be taken in performing this test to see that the original solutions are dilute; if strong solutions are used all the carboxyhaemoglobin may not be decomposed and a pinkish coagulum of carboxyhaemochromogen is formed.

In addition to the above mentioned compounds the following derivatives may also occur.

HAEMATOIDIN an iron free derivative of haemoglobin which crystallizes in bright orange red plates, and occurs in old blood extravasations, thrombi and aneurisms. Crystals have been found in the urine after blood transfusions and in cases of icterus where there is destruction of red corpuscles. It shews no spectrum.

HAEMOSIDERIN is found in extravasations in the living organism. It differs from haematoidin in containing iron.

KATHAEMOGLOBIN (Neutral Haematin) is a pigment which Arnold who first obtained it called neutral haematin. It is obtained when methaemoglobin prepared with potassium ferricyanide is shaken with chloroform. A lightred brown precipitate forms which contains the whole of the blood pigment, and shews three absorption bands at 606, 546, and 532.

ALCOHOL IN THE BLOOD.

Owing to the recent increase in motor accidents where the drivers have been charged with drunkenness, the possibility of the determination of alcohol in the blood, as an index of the degree of intoxication, has arisen.

The amount of alcohol in the blood varies very closely with the amount excreted in the urine, and an estimate of the latter gives a fairly accurate idea of the amount in the blood. For convenience, the method adopted is the estimation of alcohol in the urine.

The method used is a modification of Cannan and Sulzer's (106) method for the estimation of the alcohol in blood. Carter and Southgate (107) in this country, and Bogen (24) in Ohio have used this method in their investigations.

In my opinion the only conclusions which can be drawn from this estimation are that the individual has recently imbibed alcohol, or possibly a considerable amount. Any estimation of the degree of intoxication by this means would be fallacious. Various authors are disposed to attach considerable importance to this test, the method of estimation is therefore given below.

ESTIMATION OF ALCOHOL IN THE URINE (After Southgate)

The urine is evaporated slowly at 80°C in a current of air previously washed through concentrated sulphuric acid.

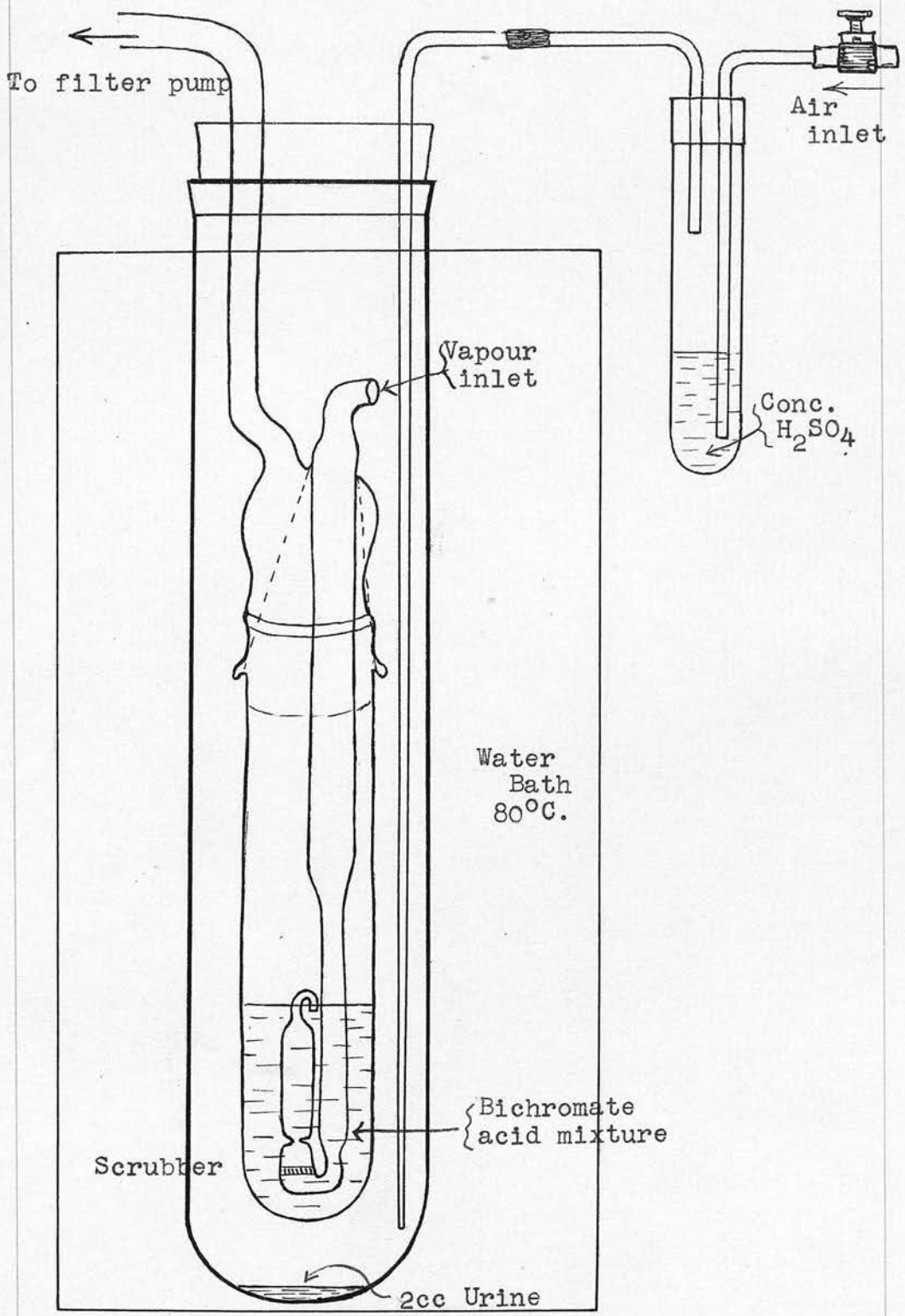
The mixture of air and alcohol vapour is led through a solution of potassium bichromate (15cc N/5) and strong sulphuric acid (20cc) in a specially designed apparatus.

The alcohol is oxidized to acetic acid at the expense of some of the bichromate. The unreduced bichromate is estimated by allowing it to liberate its equivalent of iodine from an excess of potassium iodide , the liberated iodine being titrated with a standard solution of sodium thiosulphate

1cc N/5 bichromate solution is equal to 0·0023 grammes of alcohol.

The remaining bichromate is estimated by adding to the acid solution excess of potassium iodide (2 grammes in 500 cc)when a quantity of iodine, equivalent to the bichromate present, is liberated. N/10 sodium thiosulphate is now run in, and as the equivalent amount of thiosulphate enters the free iodine combines and the brown colour disappears. When the colour has almost gone starch solution is added to make a more visible endpoint.

As N/10 sodium thiosulphate solution is half the strength of N/5 bichromate,, half the number of cc's thiosulphate will be the quantity of unreduced bichromate solution. This subtracted from the original volume gives the amount of bichromate reduced by the alcohol; and as 0·0023 grammes of alcohol correspond to each cc. the total in the sample of urine is obtained.



SOUTHGATE'S APPARATUS. Estimation of Alcohol in Urine.

Fig. 19.

APPENDIX "A"

The increase in the number of cases of carbon monoxide poisoning in recent years has become so great that problems arising in connection with these cases are an everyday occurrence. (Kerr 72).

The diagnosis has already been considered (p), the method of estimation, percentage in the blood required to produce symptoms, rate of absorption and also of elimination will now be considered.

METHODS OF ESTIMATING PERCENTAGE OF CO IN BLOOD

The percentage of carbon monoxide in the blood may be estimated either (1) by extracting with a vacuum pump or, by liberating and estimating the liberated gases. (2) by calorimetry or, (3) spectroscopically.

It is essential that in a Forensic Laboratory where estimations are not being made daily, the methods used should be one that is rapid, and in which the apparatus is always ready for use.

The methods used in the first class do not fulfil these requirements and they have no special advantages which recommend them for medico-legal work. Such methods are those of Harrington and Van Slyke (83), Nicloux (84) or Graham (85), a full description of which is given in the Journals referred to.

ESTIMATION BY CALORIMETRY.

The two meyhods which should be mentioned are Haldane's carmine method (86,25) and the pyro-tannic acid method employed by the United States Bureau of Mines.

THE PYRO-TANNIC ACID METHOD.

This method has been elaborated by Yant and Sayers (89), and Jones (90),of the United States Bureau of Mines, with a view to producing a method giving accurate results which is at the same time compact, rapid, and simple in operation. The apparatus which they have designed,they suggest,is well suited for Coroner's and allied work.

The principle of the method is that when tannic acid is added to a solution of blood, a grey brown precipitate forms on standing,whereas, if the blood contains COHb the precipitate is carmine red.Naturally the colour resulting from mixtures of varying proportions of oxy- and carboxyhaemoglobin will vary,and by matching the precipitate with permanent standards,an approximate (to 5 per cent) estimation of the percentage can be made.

Yant and Sayers give instructions for the making of standards from blood,but as these only last 14 days, the advantage of the method over various others mentioned, in which there is considerable preliminary preparation is lost, if these are employed. Permanent

standards may be made with artists oil colours and graded by trial and error, a very tedious operation. These permanent standards and also the complete apparatus are made commercially by the Mine Safety Appliances Company, Braddock Avenue, and by Thomas, Boulevard, Pittsburgh. Pa. U.S.A.

The actual colours used in obtaining the standards being (Welser's artists'oil colours) 1). Permallsa. 2). Ivory black 3). Alizarin crimson. 4). Raw Umber. 5)- Burned Umber. 6) Indian red and 7). Cadmium red.

Procedure. A small puncture wound is made in the finger, and .1 cc is drawn into a pipette, after removing any blood on the top of the pipette, it is placed in a bottle of distilled water, and water drawn up to the 2 cc mark, giving a dilution of 1 in 20, The blood solution is then discharged into a test tube similar to those containing the standards. Approximately .04 gram of mixture of equal parts by weight of tannic and pyrogallic acids is then added. This is the amount found to be most suitable for 2 cc of a 1 in 20 solution of blood.

The mixture may be kept in solid form as tablets or capsules, in powder form in a bottle with a measuring spoon holding .94 gram, as in the equipment used by the Bureau of Mines, or in solution either dilute or concentrated. Solutions deteriorate quickly and must be made fresh every day. The tube is next inverted

several times to ensure thorough mixing and is then allowed to stand for fifteen minutes at room temperature, after which it is compared with the standards.

If CO is indicated the tube should be allowed to stand a further fifteen minutes before a final reading is made. The observations are best made in daylight but not in direct sunlight.

Haldane's Carmine Method.

This method which was originally used by Haldane and Lorrain Smith is described in great detail by Haldane (Jour Physiol.309.Vol 44.1912) to which reference should be made. Whilst no doubt more accurate than the pyro-tannic method, it takes much longer and owing to its nature is more liable to be influenced by slight changes in the blood on standing (methaemoglobin).

In the pyro-tannic method this does not come into play to such an extent , as any methaemoglobin formed is converted into haematin in the same way as if it had been oxyhaemoglobin.

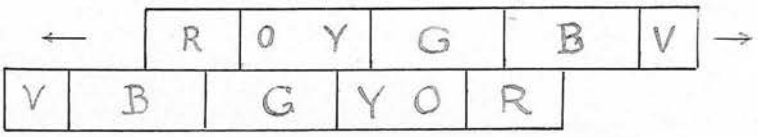
Unless the blood is quite fresh, the carmine method does not appear to me to be the method of choice, but I have not as yet, carried out comparative experiments.

ESTIMATION BY SPECTROSCOPE

This is by far the most satisfactory method for use in a Forensic Laboratory. The two bands composing the spectrum of carboxyhaemoglobin are very similar to those of oxyhaemoglobin, but are slightly nearer the purple end of the spectrum. In any mixture of oxy and carboxyhaemoglobin, the greater the amount of carboxy-haemoglobin present, the further are the bands of oxy-haemoglobin displaced towards the purple.

Balthazard (91) measures the position of the bands and having previously ascertained their position in known mixtures of oxy-and COHb, is able to calculate the percentage of CO present. In the Hartridge reversion spectroscope (92) the principle that the amount of deflection of the bands towards the purple varies with the percentage of carboxyhaemoglobin, is made use of. The amount of deflection being estimated by what is known as, the coincidence method.

The spectroscope is designed so that two similar spectra are formed lying side by side and reversed in direction to one another.



By means of a micrometer screw, one spectrum may be moved so that it is possible to make one band in the fixed spectrum coincide with the corresponding band in the movable spectrum. The amount of movement

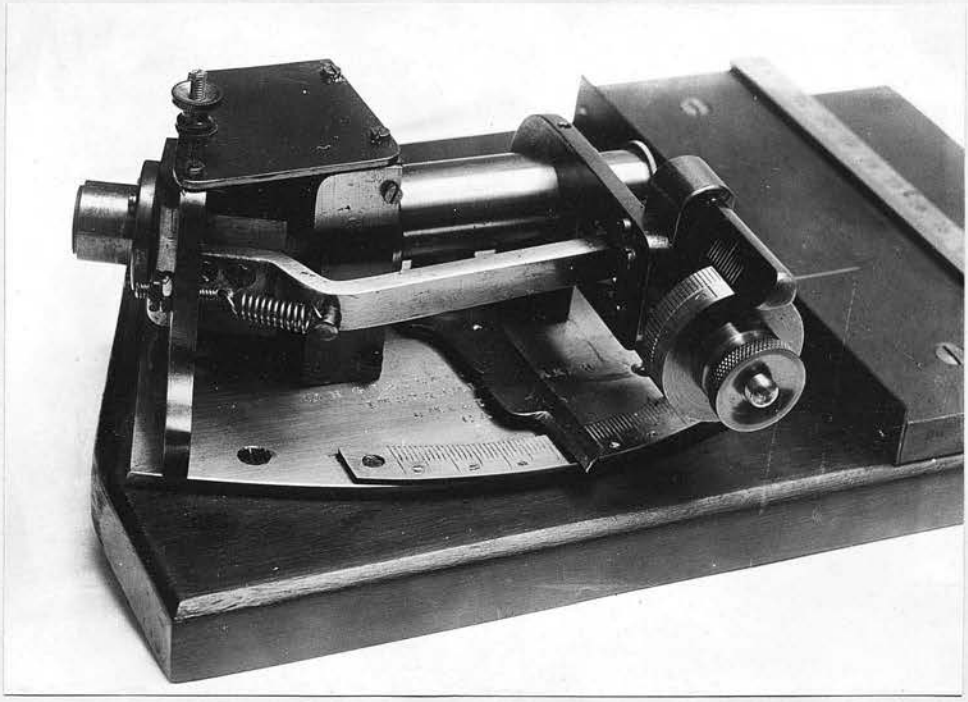


Fig. 20. Hartridge's Reversion Spectroscope.

required to do this, as indicated on the micrometer drum, varies with the position of the bands and indicated the percentage concentration of CO. The concentration corresponding to the reading on the drum having been previously determined by calibrating the instrument with solutions of known strength. The amount a band is deflected in one spectrum is the same as that in the other, and as the spectra are reversed, it follows that the amount of movement required to make the two bands coincide, is twice the amount through which the band in one spectrum has moved, This greater movement tends to greater accuracy. Hartridge (93) states that duplicate sets of readings obtained by two observers using different instruments were seldom found to differ by more than one per cent. I have not contrasted my results with those of another observer, but I find the instrument very satisfactory in practice.

Method of using the instrument

The construction of the instrument is fully detailed in the papers referred to above which should be consulted. The calibrations of the instrument with solutions of known strength is also described. The general principles of this are however, indicated below.

Calibration. Into one side of a double wedge shaped trough, (see fig below) is placed a solution of oxy-haemoglobin, into the other side is placed blood saturated with CO and of the same strength as the

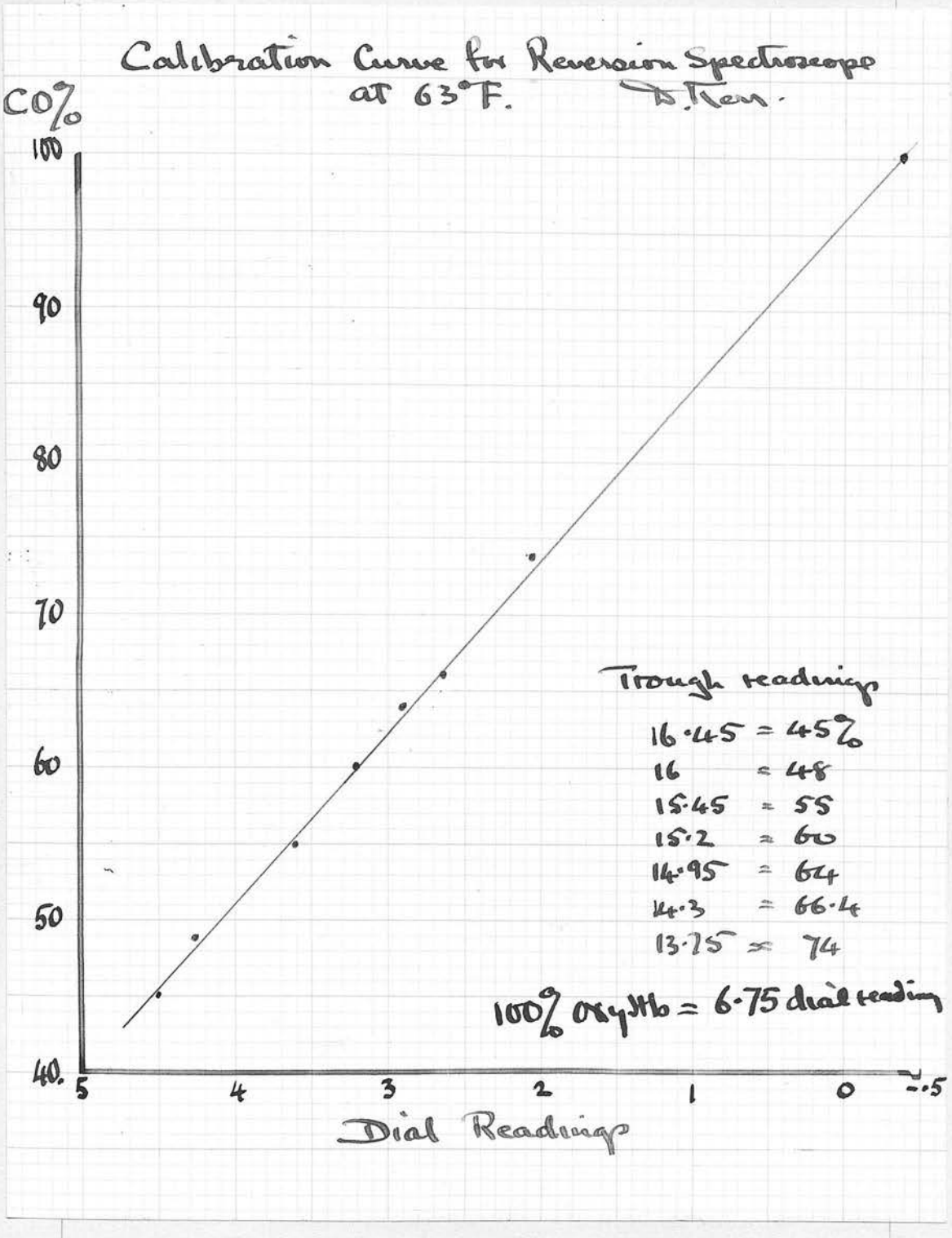
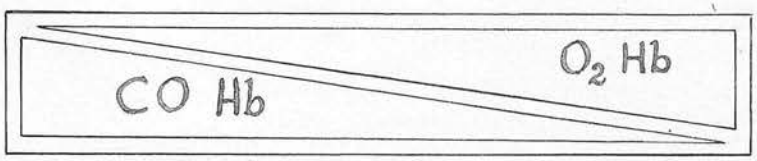

Calibration Curve for Reversion Spectroscope
at 63°F.
D. Ker.
CO%
100
90
80
70
60
50
40.
5
4
3
2
1
0
-.5
Dial Readings
Trough readings
16·45 = 45%
16 = 48
15·45 = 55
15·2 = 60
14·95 = 64
14·3 = 66·4
13·75 = 74
100% OxyHb = 6·75 dial reading

Fig. 21.

oxyhaemoglobin.



By moving the trough, the proportions of CO to oxy-Hb can be changed, and the corresponding reading on the drum noted. These readings are plotted as indicated in the diagram . From the graph so formed the percentage of CO indicated by the readings on the micrometer drum can be at once be read off. The graph for readings from about 45 per cent CO upwards is a straight line, amounts of CO below this are not of much forensic interest and the readings corresponding to these smaller amounts have not been plotted; the graph however, becomes curved as the amount of CO present falls and so a greater number of points must be determined.

On the careful construction of this calibration table depends the accuracy of future estimations, and each individual should construct his own curve. Once constructed, future estimates are a matter of minutes.

Making an estimation

A large drop of the blood to be tested is sucked up into a pipette and diluted about 1 in 200 with water, The actual dilution depending on the observer. The solution is then placed in a small glass cell in

front of the slit of the spectroscope, and the two α bands (bands nearest the red) are made to coincide the drum reading is noted. Ten such readings are made and the mean taken as correct. By referring to the calibration curve the percentage of COHb present is at once seen, the whole proceeding taking two or three minutes.

It should be remembered that the bands of nitroxy-haemoglobin are nearer the red than those of carboxy-haemoglobin, so that this method of estimating the amount of CO, automatically excludes the fallacy of nitroxyhaemoglobin.

The reversion spectroscope is the best method for determining the amount of CO in the blood. It is very rapid, requires a very small amount of blood and is always ready for use. It is the ideal instrument for a Forensic Laboratory. The instrument is now supplied commercially by W.H.Grayling, instrument maker. 8 Milton Road, Cambridge.

APPENDIX "A"

PERCENTAGE SATURATION OF BLOOD REQUIRED TO PRODUCE UNCONSCIOUSNESS

The percentage saturation of carbon monoxide in the blood required to produce symptoms varies with the individual and with the rapidity with which the saturation is produced.

40 per cent produces slight palpitations and great weakness with exercise.

45 per cent, similar symptoms after slight movements. 50 per cent, uncertainty in walking, feeling of confusion. 55 per cent, patient can hardly stand and cannot walk about.

The symptoms are dur to diminution in the oxygen carrying power of the blood, and as will be seen from the above figures, unconsciousness will supervent between 55 to 60 per cent, though a slight degree of tolerance may be acquired.

The percentage saturation required to produce symptoms is less when the saturation has been produced rapidly, as when breathing high contratines of the gas.

APPENDIX "A"

RATE OF ABSORPTION OF CARBON MONOXIDE BY THE BLOOD

Yandell Henderson (74) considers the accurate determination of the time required before a given saturation of a gas is reached in the blood under laboratory conditions. In medico-legal work such accuracy is not possible, nor desirable. The maximum amount of gas which can be absorbed by the blood depends on its concentration in the inspired air (its partial pressure) and is therefore a variable quantity

Though carbon monoxide is a cumulative poison, if the concentration in the atmosphere is too low, the point of equilibrium or maximum saturation of the blood for that concentration may be too low to produce unconsciousness or even symptoms. Haldane states that the maximum saturation of the blood for any given concentration of CO in the air, is reached within two and a half hours. He shewed (15) that·2 per cent of CO in the atmosphere produced a concentration of 50 per cent in the blood in seventy minutes.

When air containing CO is breathed, about half the CO is absorbed. A man's blood will take up 1 litre of O or CO. So to produce a concentration of 55 per cent, 550 cc of CO must be inhaled. A man breathes 7 litres of air per minute, so, if the air contains 1 per cent of CO 70 cc of CO will be breathed per minute, half the CO breathed is absorbed, so with 1 per cent of CO in the air, a man absorbs 35 cc per minute. The higher

/over

the concentration of CO in the blood, the slower will be the absorption, as there is less blood free to absorb the CO, but until the concentration rises above about 50 per cent, an approximate idea of the time taken may be obtained by simple proportion assuming that,

1 per cent of CO gives 35 cc of CO in the blood in 1 minute,

that, is 1 per cent of CO gives 35 per cent saturation in the blood in 1 minute.

Using this formula, 2 per cent CO in the blood would give 49 per cent saturation in 70 minutes. Haldane found by experiment that the actual saturation was 50 per cent. The actual time required for unconsciousness depends in cases of poisoning by domestic gas supply, on how near to the gas jet the individual may be, that is, how great is the concentration?.

In cases where the head has been confined, or the gas tube placed in the mouth, almost pure coal gas is breathed. This contains in Edinburgh, 21 per cent CO, under these circumstances(55 per cent saturation) unconsciousness would theoretically take place in under half a minute.

RATE OF ELIMINATION

Within a few hours of profound but not fatal poisoning by carbon monoxide, no trace of carbon monoxide can be found in the blood. The victim may however, die within the next few days from cerebral softening. For details of the histo pathology, the

reader is referred to an article by Stewart (J. Neur. and Psychopathol. Aug. 1920).

With saturations up to about 50 per cent about one half of the carbon monoxide absorbed is eliminated in the first hour.

When the saturation exceeds 50 per cent and coma is present, the breathing is small in amount, and the elimination during the first hour is very slow.

Henderson experimented with dogs, and found with blood saturations of 60 per cent very slow elimination for the first hour, but after that the rate increased and in two hours and twenty minutes the blood saturation was reduced to 10 per cent.

The following diagram indicates his results;

Fig. 22.

BIBLIOGRAPHY

* Starred items were not consulted in the originals.

1. Roaf Textbook of Physiology.
2. Hammarstein Textbook of Physiology.
3. Milroy Practical Chemical Physiology.
4. Lloyd Guy's Hospital Reports 1924 376.
*5. Dittrich Arch. f.Exp.Path.u.Pharm.1892, 247.
6. Gordon Watson B.M.J. 1925. ii.1049.
7. Kerr D.J.A. B.M.J.1925.ii. 1199.
8. Haldane B.M.J. 1925. ii. 1199.
9. Sutherland Blood Stains.
10. Dilling Erklärung zu den Spektraltäfeln &c. 1911
*11.Jüderholm Nord. med. Arkio. 16.
12. Halliburton Textbook of Physiology.
13. Hawke Practical Physiol. Chem. 7th Ed.
*14. Skand Arch. f. Physiol. 3.
15. Garrod Journ.of Physiol. 13.
16 Haldane J.of Physiol. 1900 (Feb 25).
17. Lyon and Waddell Med.Jurisp. for India. 1914.
18. Haldane,Makgill and Mavrogardato J of Phys. 1897
19. Haldane and Banham B.M.J. 1925.ii.
20. Flack and Hill Textbook of Physiol. 1919.
21. Anson and Mirsky J.of Physiol.1925.
22. Henderson Y. B.M.J. 1926,i.
*23 Kostin Pflüger's Arch. 84.
24. Bogen Calif.& West.Med. 1927,June.
25. Haldane J. of Physiol. 1895, 465.

26. Haggard H.W. J.of Ind.Hyg. 1925, vii.113.

27- Mackenzie Wallis Q.J.of Med. 1913, 77.

28. Van der Bergh Deut. Arch.f.Klin.Med. 1906.

29. Wood Clarke &&W.H.Hurtley J.of.Phys.xxxvi,62

*30. Hoppe Seyler Med. Centralblatt 1863.

31. Garrod Quart. J of Med. 73. 1925, 86.

*32. Klaverev V. Zeit.f.Physiol.Chem.33.

*33. Nenki & Sieber Ber.d.Deut.Chem.Gesell. 18.

*34. Ludwig Du Bois Raymond Arch.1890.

35. Stitt Pract. Bacter.Blood & Parasit. 6th Ed.

36. Kerr & Mason Haemoch.Test for Blood B.M.J.1926

37. Beam & Freak Biochem.J.ix, 1925 March.

38.

39. Takayama Jap. J.of Toxicol. 1912.

40. Mahler.K. Deut.Zeit.f.d.Ges.Ger.Med 1923,671.

41. Nippe Deut.Med.Woch. 1912.2222

*42. Katayama Viertel.f.Gericht.Med- 1888.269.

*43. Ferrai.C. Boll.R.Accad.di med.d.Genova 1901.272

*44. Biondi Viertel.f.ger.Med.1892 Supp.1.

45. Sorby Woodman & Tidy For.Med.587.

*46. Tamarsia At.del R.ist.vento d.Sci.Let.Art.1883

47. Taylor Medical Jurisprudence.

48. Woodman & Tidy Textbook of Legal Medicine.

49. Hammerl Viertel.f.ger.Med. 1892 iv 44.

50. Petterson & Haines Leg.Med & Toxicology 1923.

51 Copeman B.M.J. 1889.ii.190.

52. Reichert and Brown Carnegie Inst.Pub.116. 1909.

53 Glaister Textbook of Forensic Medicine.

54. Buchanan Textbook of Forensic Medicine 9th Ed.

55 Glaister Jun. B.M.J. 1926.i.

56 Rabinovitch Arch.di Krimin.et d.Méd.lég.1926.

57. Kastle Bull.S. I. Hyg.Lab.1909.

58 Boas Deut.Med.Woch. 37. 1911.62.

59. Kerr D.J.A. B.M.J. 1926.i.

60. Hartridge Proc.Roy.Soc. 1913. 128.

61. Jüderholm Nord.Med.Ark. 16.

62. Haldane Journ. of Hyg 1901. 115.

63. Gamgee Schaefer's Physiol. Vol i. 1898.

64. Balthazard Méd.Légale Paris 1911.

65. Douris Guide Pract.pour l'analyse du Sang 1925.

66. Haldane Journ of Physiol 1898.Chemist.of Hb.

67 Anson & Mirsky Jour.of Physiol. 1925. p100.

68 Hartridge J. of Physiol. 1912. p22.

69. Anson & Mirsky Jour.of Physiol. 1925 p50.

70. Balthazard & Martin Ann.de Méd. Légale 1926.

71. De Dominicis I Cristalli di emocromogemo. Milan1902

72. Kerr D.J.A. Carbon Monoxide Poisoning B.M.J.1926.

73. Hamilton Agnes Industrial Poisons.Macmillan 1925

74. Henderson Y. Elimination of Volatile &c. B.M.J.1926. i.

75. Haldane Journ.of Physiol.1895.

76. Graham Smith & Sawyer Journ.of Hygiene 1903.273.

77. Dean & Webb Jour. of Path.& Bact. xxix 1926.

78. Kolmer Immunity and Specific Therapy. 1915.

79. Nuttall Blood Immunity & Relationship Tests. 1904.

80. Hektoen & Schulhof J.of Inf.Dis. 1923.224.

*81. Manoiloff Münch. Med. Woch. 1925.ii.2186.

*82. Manoiloff Zeit f.d.Ges.Gericht. Med 1924. 315.

83. Harington & Van Slyke J.of Biochem. Sept.1924.

84. Nicloux Bull. Soc. Chem. Biol.1920.

85. Graham Jour.of.Soc of Chem Indust. Jan.1919.

86. Haldane Jour. of Physiol. 1912.

*87. Zaugger H. Vergiftungen. Leipzig 1924.

*88. Laet,Maurice de R.de Droit Pen.et de Droit Crim. 1924.32, abst. Zeit. f.Ges.Ger.Med.1925.486.

89. Sayers & Yant U.S.A.Dept.of Commerce No373.1925.

90. Sayers,Yant & Jones U.S.A.Health Report No 872.1923

91. Balthazard Ann.de Méd. Légale 1924

92. Hartridge Proc.Roy.Soc. Vol A.102. 1923.

93. Hartridge Jour.of Physiol. 1923 Dec.

*94. Hajek Arch f. Mikr.u. Entom. 101.1924.

95. Goroncy Zeit.f.Ges.Ger.Med. 1925.178.

96. Schiff Technik der Blutgruppenuntersuchung 1926.

97. Lattes Individualität des Blutes 1925.

98. Reinheimer Zeit f.Ges.Ger. Med. 1926. 560.

99. Snyder J.A.M.A. 1927. ii.

*100. Bernstein Klin. Woch 1924.p.1924. Zeit.f.ind. Abstamm.u.Vererbungslehre 1925, 237.

101. Strassmann Deut. Zeit.f.Ges.Ger.Med 1925. 184

*102. Thomsen Hospitalstidende Copenhagen 1926 Dec.

103. Roche Trans.Med.Legal Soc. 1924 Jan.

104. Goroncy Zeit.f.Ges.Ger.Med 1926.

105. Sydney Smith Textbook of Forensic Medicine.

106. Cannan & Sulzer Heart xi 1924 Feb.

107 Carter & Southgate B.M.J.1926 i.464